ADOLESCENT TYPE 1 DIABETES EATING AND GASTROINTESTINAL FUNCTION

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To God Be the Glory
Andrae Crouch

To Magnus, Axel, and our coming baby
ABSTRACT

Adolescents with type 1 diabetes (T1DM) are given nutritional education, but the knowledge about their adherence to the food recommendations and associations between dietary intake and metabolic control is poor. Gastrointestinal symptoms are more prevalent in adults with T1DM than in healthy controls, which may be due to disturbed gastrointestinal motility. The meal content affects the gastric emptying rate and the postprandial glycaemia in healthy adults and adults with type 2 diabetes. Meal ingestion also elicits several postprandial hormonal changes of importance for gastrointestinal motility and glycaemia. Eating disorders are more prevalent in young females with T1DM than in healthy females, and are associated with poor metabolic control. The prevalence of eating disorders in adolescent boys with T1DM is not known.

This thesis focuses on eating and gastrointestinal function in adolescents with T1DM. Three population-based, cross-sectional studies demonstrated that adolescents with T1DM consume healthy foods more often and have a more regular meal pattern than age- and sex-matched controls. Yet both boys and girls are heavier than controls. The intake of saturated fat is higher and the intake of fibre is lower than recommended in adolescents with T1DM. Patients with poor metabolic control consume more fat and less carbohydrates than patients with better metabolic control. Gastrointestinal symptoms are common in adolescents with T1DM, but the prevalence is not increased compared with controls. Gastrointestinal symptoms in patients are associated with female gender, daily cigarette smoking, long duration of diabetes, poor metabolic control during the past year, and an irregular meal pattern. Adolescent boys with T1DM are heavier and have higher drive for thinness than healthy boys, but do not differ from them in scales measuring psychopathology associated with eating disorders.

In a randomized, cross-over study, we found that a meal with a high fat and energy content reduces the initial (0–2 hours) postprandial glycaemic response and delays gastric emptying in adolescents with T1DM given a fixed prandial insulin dose compared with a low-fat meal. The glycaemic response is significantly associated with the gastric emptying rate. Both a high- and a low-fat meal increase the postprandial concentrations of glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) and suppress the postprandial ghrelin levels in adolescents with T1DM. The postprandial changes of these hormones are more pronounced after the high-fat meal. Insulin-like growth factor binding-protein (IGFBP) –1 concentrations decrease after insulin administration irrespective of meal ingestion. The GLP-1 response is negatively associated with the gastric emptying rate. The fasting ghrelin levels are negatively associated with the postprandial glycaemic response, and the fasting IGFBP-1 levels are positively associated with the fasting glucose levels.

We conclude that nutritional education to adolescents with T1DM should focus more on energy intake and expenditure to prevent and treat weight gain. It should also focus on fat quality and fibre intake to reduce the risk of macrovascular complications and improve glycaemia. Gastrointestinal symptoms in adolescents with T1DM should be investigated and treated as in other people irrespective of having diabetes. However,
adolescents with long duration of diabetes, poor metabolic control, and symptoms from the upper gut should have their gastric emptying rate examined during euglycaemia. There may be an increased risk for development of eating disorders in adolescent males with T1DM since they are heavier than healthy boys and have higher drive for thinness. This should be investigated in future, larger studies.

For the first time, we showed that a fat-rich meal delays gastric emptying and reduces the initial glycaemic response in patients with T1DM. The action profile of the prandial insulin dose to a fat-rich meal may need to be postponed and prolonged compared with the profile to a low-fat meal to reach postprandial normoglycaemia. Circulating insulin levels affect postprandial GIP, GLP-1, and ghrelin, but not IGFBP-1, responses less than the meal content. The pronounced GIP-response to a fat- and energy-rich meal may promote adiposity, since GIP stimulates lipogenesis. Such an effect would be disadvantageous for adolescents with T1DM since they already have increased body fat mass and higher weights compared with healthy adolescents. Adolescents with T1DM may have subnormal postprandial ghrelin suppression, which may be due to their increased insulin resistance or elevated growth hormone levels. This needs to be investigated in future, controlled studies.
LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. M. Lodefalk, J. Åman. Food habits, energy and nutrient intake in adolescents with Type 1 diabetes

II. M. Lodefalk, J. Åman. Gastrointestinal symptoms in adolescents with Type 1 diabetes
    *Submitted to Pediatric Diabetes*

III. M. Lodefalk, J. Åman, P. Bang. Effects of fat supplementation on glycaemic response and gastric emptying in adolescents with Type 1 diabetes

IV. M. Lodefalk, C. Carlsson-Skwirut, J.J. Holst, J. Åman, P. Bang. Effects of fat supplementation on postprandial GIP, GLP-1, ghrelin, and IGFBP-1 levels in adolescents with Type 1 diabetes
    *Submitted to Hormone Research*

V. M. Svensson, I. Engström, J. Åman. Higher drive for thinness in adolescent males with insulin-dependent diabetes mellitus compared with healthy controls

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1 My surname was Svensson before I married in July 2003.
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<tr>
<td>AN</td>
<td>Anorexia nervosa</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>BED</td>
<td>Binge eating disorder</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>BN</td>
<td>Bulimia nervosa</td>
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<td>CCK</td>
<td>Cholecystokinin</td>
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<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximal concentration</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CSII</td>
<td>Continuous subcutaneous insulin infusion</td>
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<td>CV</td>
<td>Coefficient of variation</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DPP-IV</td>
<td>Dipeptidyl peptidase IV</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<td>E%</td>
<td>Energy per cent</td>
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<td>ED</td>
<td>Eating disorder</td>
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<td>EDI</td>
<td>Eating Disorder Inventory</td>
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<td>EDI-C</td>
<td>Eating Disorder Inventory for Children</td>
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<td>EDNOS</td>
<td>Eating disorder not otherwise specified</td>
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<td>EGG</td>
<td>Electrogastrography</td>
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<td>Enteric nervous system</td>
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<td>Food frequency questionnaire</td>
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<td>GH</td>
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<td>GHRH</td>
<td>Growth hormone releasing hormone</td>
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<td>Growth hormone secretagogue</td>
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<td>GI</td>
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<td>GIP</td>
<td>Glucose-dependent insulino tropic polypeptide</td>
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<td>Glucagon-like peptide 1</td>
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<td>Glucagon-like peptide 1 receptor</td>
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<td>HbA1c</td>
<td>Glycated hemoglobin</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<tr>
<td>ICC</td>
<td>Interstitial cells of Cajal</td>
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<td>IGFBP</td>
<td>Insulin-like growth factor binding-protein</td>
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<tr>
<td>ISPAD</td>
<td>International Society for Pediatric and Adolescent Diabetes</td>
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<td>IU</td>
<td>International units</td>
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<tr>
<td>Iv</td>
<td>Intravenous</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<td>MDI</td>
<td>Multiple daily injections</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>Sc</td>
<td>Subcutaneous</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>Standard deviation score</td>
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<td>Description</td>
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<tr>
<td>SNR</td>
<td>Swedish Nutritional Recommendations</td>
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<tr>
<td>T1DM</td>
<td>Type 1 diabetes mellitus</td>
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<td>T2DM</td>
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1 INTRODUCTION

1.1 TYPE 1 DIABETES IN CHILDREN AND ADOLESCENTS

Type 1 diabetes (T1DM) is the second most common chronic disease in childhood. The reported incidence in Sweden is, after that in Finland, the highest in the world. It has doubled the last 25 years and was in children 0–15 years 43/100.000 in 2005 [1]. The prevalence in Sweden was approximately 4/1000 children 0–15 years in 2005 [1]. T1DM constitutes about 98% of all diabetes in children and adolescents in Sweden.

The etiology is multifactorial. A genetic predisposition exists, but is complex. Environmental factors are thought to be responsible for the increasing incidence. Such factors seem to include exposure to certain viral infections, dietary antigen, increased growth rate, physiological and psychological stress, and changes in frequency of infections. The pathogenesis involves an autoimmune inflammation in pancreatic β-cells leading to their destruction and subsequently diminished and finally ceased insulin production.

A cure for T1DM has not yet been found. Treatment of the disease involves the administration of exogenous insulin, either by subcutaneous (sc) injections or by a continuous sc infusion (CSII) using an insulin pump. Conventional treatment means one or two daily insulin injections, includes often premixed insulin (both long and short acting in one shot) and was used frequently earlier. Today most patients with T1DM in Sweden are treated by multiple daily insulin injections (MDI), which means about three to six injections each day and include basal long-acting insulin (or insulin analogue) once or twice a day and rapid-acting insulin analogue before each meal and snack. The aim of the treatment is normoglycaemia to reduce the risk of acute and long-term complications. To reach normoglycaemia, the patients and their parents need to constantly consider the factors that influence glycaemia, i.e. food intake, physical activity, dose of insulin given, illness or other conditions that induce insulin resistance, time of the day, and other things. The patients and their parents monitor the plasma glucose concentration repeatedly every day and are taught how to adjust the insulin dose accordingly. Thus, T1DM affects everyday life and is associated with a major burden for the patient and his family.

Acute complications are mainly severe hypoglycaemia and ketoacidosis, life-threatening conditions. Long-term complications are micro and macrovascular diseases, as well as neuropathy. Microvascular complications include retinopathy and nephropathy. Fifteen per cent of all children and adolescents with T1DM in Sweden had some form of retinopathy in 2007, and as much as 20–25% of patients aged 17–19 years had retinopathy [2]. Neuropathy includes sensory and motor neuropathy, as well as autonomic neuropathy, for example delayed gastric emptying. There is also an increased prevalence of other autoimmune diseases, for example coeliac disease and hypothyroidism, and of psychological disorders, such as depression and eating disorders (EDs). In addition to this increased co morbidity, T1DM is associated with increased mortality.
1.2 DIETARY INTAKE AND TYPE 1 DIABETES IN ADOLESCENTS

Nutritional counseling is one of the cornerstones in the treatment of T1DM. To obtain normoglycaemia, the food intake is of greatest importance and determines whether insulin can be adequately dosed. For example, a high intake of sugars cannot be easily controlled by exogenous insulin. Furthermore, nutritional counseling should provide skills to understand how meal compositions affect insulin needs over time taking other factors such as physical activity and insulin sensitivity into consideration. The food intake should also be balanced and healthy to reduce the risk of long-term complications, appropriate for growing children, and counteract the development of overweight. A good glycaemic control, most often measured as a near normal glycated hemoglobin value (HbA1c), is essential for minimizing the risk of microvascular and neurologic complications [3,4] either via direct glycaemic effects or possibly by keeping hormonal systems balanced. The dietary intake may play a significant role for the glycaemic control.

Adolescents with T1DM, particularly females, have higher weights and increased body fat mass compared with healthy adolescents [5-7]. Intensive insulin therapy with multiple insulin doses and improved glycaemic control may lead to weight gain and increase in body fat mass [8,9], which may be due to anabolic effects of insulin on fat metabolism. Current intake of dietary fat is associated with the one-year change in body fat mass in adolescent girls with and without T1DM [10]. Reduced physical activity because of fear of hypoglycaemia and increased food intake to cope with hypoglycaemic episodes may also lead to weight gain. Thus, the dietary intake is of importance for body weight and body composition in adolescents with T1DM.

Food recommendations for patients with T1DM have changed during the last decades and the scientific evidence behind them is weak [11]. Current food recommendations for children and adolescents with T1DM are not different from recommendations for healthy individuals. The total daily energy intake should be distributed as 50–55% carbohydrates, 30–35% fat, and 10–15% protein. The sucrose intake and the intake of saturated fat and trans fatty acids should not exceed 10% each of the total daily energy intake and the fibre intake should be 2.8–3.4 g/MJ [12].

All paediatric patients with T1DM in Sweden are treated by health professionals working together in teams. These teams include dieticians who teach patients and their families about nutrition, dietary recommendations, and how to achieve the dietary goals. The recommendations given are in accord with international recommendations and the education focuses on healthy eating habits using the plate model [13]. The patients are encouraged to have a regular meal pattern, use a consistent baseline insulin dose, and frequently monitor their plasma glucose levels. They learn to recognize patterns of plasma glucose responses to nutrient intake and to adjust their prandial insulin dose according to pre-meal plasma glucose level, nutrient intake, and physical activity. This education level corresponds to the second of three identified levels of carbohydrate counting [14]. Counting carbohydrates (in grams) and using insulin-to-carbohydrate ratios, the third education level, are not common in Sweden today, nor is the use of the exchange or portion system [12].
The use of an insulin-to-carbohydrate ratio may be appropriate for patients with MDI or insulin pump therapy. It involves the estimation of carbohydrates (in grams) that the patient is planning to eat and the calculation of the prandial insulin dose supposed to be needed for that quantity of carbohydrates. The insulin dose needed for a certain quantity of carbohydrates, the insulin-to-carbohydrate ratio, is dependent on the patient’s age, sex, pubertal status, duration of diabetes, time of the day, and physical activity [12]. The method has not been evaluated in children and adolescents with T1DM yet, but has been shown to improve metabolic control, dietary freedom, and quality of life in adults with T1DM [15]. However, estimation of the carbohydrate content in meals is hard to perform properly and does not take into account that different sorts of carbohydrates have different effects on postprandial glycaemia and that other nutrients than carbohydrates, particularly fat, may influence postprandial glycaemia as well. There is also a risk that quantifying carbohydrates leads to carbohydrate restriction and psychological problems, arguments that would favour qualitative carbohydrate education as more appropriate [11].

The glycaemic index is a method for describing the plasma glucose increasing effect of different carbohydrates in a systematic way [12]. A carbohydrate with a high glycaemic index will increase postprandial glycaemia more than an equal quantity of a carbohydrate with a low glycaemic index. The glycaemic load also takes into account the quantity of the carbohydrates.

The knowledge about the dietary intake in adolescents with T1DM has been poor, but recently a few large studies have been published [16,17]. Of the earlier studies, the one by Virtanen et al. is the most relevant [18], but it describes the dietary intake 24 years ago. Then Finnish adolescents (11.7–17.3 yr) with T1DM consumed more protein and less fat and sucrose than healthy adolescents and the diet of the patients was in accord with food recommendations given then, except for a slightly higher intake of sucrose [18]. The fibre intake was much higher in adolescents with diabetes compared with controls [19]. However, the differences between diabetic patients and controls decreased or disappeared with age [18].

A more recent study reports that American adolescents (10.7–14.2 yr) with T1DM eat more fat and protein, but less carbohydrates, than healthy controls and more saturated fat and less fibre than recommended [17]. Another American study finds a higher intake of both total and saturated fat, and a lower fibre intake than recommended in youth (10–22 yr) with T1DM [16].

Our knowledge about the impact of the dietary intake on glycaemic control in children and adolescents with T1DM is still poor. It is based on a few randomized intervention studies and some cross-sectional studies looking at associations between reported intake and measured metabolic control. Dietary intervention studies are unfortunately extremely difficult to perform, mostly because of problems with adherence to a prescribed test diet or a control diet and difficulties in objective ways to measure actual intake. In addition, long-time interventions and large study samples may be required to detect significant effects of a diet. Cross-sectional studies can never prove causality. They can only show correlations.
However, randomized, controlled intervention studies have shown that a high intake of soluble fibres improves glycaemic control and reduces serum total cholesterol levels in children and adolescents with T1DM [20,21]. Children with T1DM given flexible low glycaemic index dietary advice have after one year lower mean HbA1c value than children with T1DM given measured carbohydrate exchange diet advice [22].

Studies on associations between dietary intake and metabolic control in children and adolescents with T1DM have shown different results. An increased intake of monounsaturated fatty acids is associated with improved metabolic control and reduced plasma total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations in adolescents with T1DM [23]. A high intake of total fat [24] and saturated fat [25], a low intake of fibre [26], a low number of daily eating occasions [27], and an irregular meal pattern [26] associate with poor metabolic control. A high day-to-day variation in energy intake associates with good metabolic control in one study [27], but the opposite is found in another study [24].

The fat quality of the diet influences insulin sensitivity and the plasma lipid profile, which in turn may influence the risk of macrovascular disease. A high intake of saturated fatty acids deteriorates insulin sensitivity in adults [28] and an increased intake of polyunsaturated fatty acids reduces insulin resistance in overweight adults [29]. Although insulin resistance is not the primary defect in patients with T1DM, it is highly relevant for adolescents with this disease. Puberty is associated with increased insulin resistance, and in adolescents with T1DM it is increased even more [30]. Insulin resistance in T1DM leads to a need for higher insulin doses, increased weight, and deterioration of metabolic control [31].

The influence of the fat intake on the plasma lipid profile has been shown in studies of adults. Intervention programs aiming at a dietary intake of no more than 30 E% fat, 10 E% saturated fat, and 300 mg cholesterol decrease plasma total cholesterol, LDL cholesterol, and triglycerides levels [32], which reduce the risk for cardiovascular disease (CVD) [33,34].

A reduced protein intake reduces glomerular filtration rate, filtration fraction, and fractional clearance of albumin in adolescents and young adults with T1DM and mainly in patients with hyperfiltration on usual diet [35,36]. This may reduce the risk of diabetic nephropathy.

1.3 GASTROINTESTINAL MOTILITY AND DIABETES

Gastrointestinal (GI) motility is a complex process, which may be disturbed in patients with long-standing diabetes. Recent research has shed new light on the different parts responsible for it.

The enteroendocrine cells in the GI mucosa respond to mechanical pressure and nutrients in the intestinal lumen and signal to the enteric nervous system (ENS), which regulates most of the physiologic processes in the gut, such as motor functions, blood
flow, secretion, absorption, and modulation of the immune response against pathogens [37]. Besides different types of neurons, the ENS contains enteric glial cells, which give mechanical support to the ENS and have neurotransmitter, immune, and homeostatic functions in the gut. These cells may also have an active role in GI motility [38].

The interstitial cells of Cajal (ICC) mediate neurotransmission from the ENS to the smooth muscle cells in the gut, and they are also necessary for the pacing of the electrical slow-wave activity characteristic of the upper GI tract [39]. The smooth muscle tissue contracts and relaxes in different ways leading to specific motor patterns. Those of the upper gut include peristalsis, segmentation, migrating motor complex, and a postprandial motor pattern. The motility of the colon is irregular and complex and includes several distinct motor patterns [37].

The ENS is in close contact with the central nervous system (CNS) through extrinsic afferent and efferent pathways, which follow two major routes, the spinal and the vagal pathways. In general, vagal stimulation inhibits GI secretion and motor activity and promotes contraction of GI sphincters and blood vessels, while spinal stimulation has opposite effects [37].

The motor neurons in the ENS are either excitatory, mediating contraction, or inhibitory, mediating relaxation. The excitatory motor neurons use acetylcholine, and tachykinins, such as substance P and neurokinin A, and perhaps also galanin as neurotransmitters [37,40]. The inhibitory motor neurons use nitric oxide, vasoactive intestinal polypeptide, γ-aminobutyric acid, carbon monoxide, and pituitary adenyl cyclase-activating polypeptide as neurotransmitters [37].

There is a wide spectrum of hormones and peptides produced in and secreted from the GI tract that influence GI motility through autocrine, paracrine, or endocrine pathways, for example secretin, somatostatin, cholecystokinin (CCK), melatonin, serotonin, motilin, ghrelin, peptide YY, neuropeptide Y, glucagon-like peptide 1 (GLP-1), endocannabinoids, and orexins [41-49].

Furthermore, mood disturbances such as anxiety and depression are associated with changes in GI motility [50], however little is known about the signaling pathways.

Some histopathological changes in this complex system regulating GI motility have been found in patients with diabetes, which may lead to disordered motility and GI symptoms. Adults with long-standing T1DM and GI symptoms have abnormal density of endocrine cells in both upper and lower GI tract [51], and adults with T2DM are deficient in ICC in the colon and the stomach [52,53]. Patients with T2DM and diarrhea or constipation have a lower content of substance P in the rectal mucosa compared with patients with T2DM and normal bowel habits and compared with controls [54]. In animal models of diabetes, several changes in the ENS are described, both in morphology and function, caused by neuronal apoptosis, oxidative stress, and effects of advanced glycation end products [55].
Disordered motility of the stomach, most often described as delayed gastric emptying, is the most extensively investigated dysmotility in the gut of patients with diabetes, even though oesophageal, gall bladder, and colonic dysmotility also have been reported [56-59]. The expression “gastroparesis diabeticorum” was introduced in 1957 [60] and means a state of disordered antral peristalsis leading to delay of emptying of solid foods and symptoms such as nausea, vomiting, early satiety, bloating, and abdominal pain. Gastroparesis diabeticorum may also be asymptomatic and was earlier believed to be due to vagal autonomic neuropathy [61], but many different mechanisms behind the condition is now recognized [62]. Gastroparesis diabeticorum is typically seen in patients with long-standing, insulin-dependent diabetes that has been poorly controlled for many years and is complicated with peripheral and autonomic neuropathy, nephropathy, and retinopathy [61]. Delay in gastric emptying in adults with long-standing T1DM correlates with their degree of autonomic neuropathy [63].

Gastric emptying of solid meals is reported to be abnormally slow in 30–35% of adults with long-standing diabetes [58,64]. However, the gastric emptying rate was not measured during euglycaemia in these studies and therefore the results may be inaccurate. The gastric emptying rate is profoundly affected by the current plasma glucose concentration. Hyperglycaemia, even a physiologic postprandial glucose elevation, slows gastric emptying of both solid and liquid nutrients in both healthy individuals and in patients with T1DM [65-67]. The mechanism may involve endogenous prostaglandins [68]. Hypoglycaemia, on the other hand, accelerates gastric emptying of both solid and liquid nutrients in both healthy individuals and in patients with T1DM [69,70]. Acetylcholine seems to be important for this increase in gastric emptying, since atropine, an inhibitor of acetylcholine, abolishes it [71].

Studies investigating gastric emptying during euglycaemia have yielded conflicting results. Fifty-six per cent of adults with long-standing T2DM had abnormal gastric emptying of a mixed meal and the patients had significantly delayed mean gastric emptying rate compared with the controls [59]. In contrast, only 10% of adults with long-standing T1DM was found to have an abnormal gastric emptying of a solid meal and the mean gastric emptying rate in the patients was not different from that in the controls [72]. These conflicting results may be due to differences in the patients investigated, in the methods used, and in the choice of control subjects. The difficulty in evaluating the impact of delayed gastric emptying in patients with diabetes is further manifested by the facts that there are no controlled, population-based studies on gastric emptying in patients with diabetes and no long-term follow-up studies investigating the natural history of it.

In children and adolescents with T1DM, 26 out of 40 (65%) had delayed gastric emptying in one study, and those with delayed gastric emptying had more often electrogastrographic (EGG) abnormalities and higher HbA1c than patients with T1DM and normal gastric emptying [73]. The plasma glucose levels were not normalised before or during the investigation, but baseline levels did not differ between patients with and patients without delayed gastric emptying. The plasma glucose concentration 180 min after the start of the meal ingestion was higher in patients with delayed gastric emptying, but that difference probably reflects a consequence of the delay rather than a cause of it.
Eighty-five per cent of 172 children and adolescents with T1DM had abnormal gastric myoelectrical activity, present both pre- and postprandially, compared with 9% of controls in another study [74]. Only weak associations between current glycaemia and EGG readings were found, indicating that the EGG abnormalities in the patients were not due to current hyper- or hypoglycaemia. Thus, children and adolescents with T1DM may have disordered GI motility.

Delayed gastric emptying may lead to symptoms from the upper gut. Furthermore, it leads to delayed absorption of nutrients in the small intestine and a postponed increase in plasma glucose concentrations. Therefore, a mismatch between the action of the given prandial insulin dose and the meal-induced hyperglycaemia can occur, leading to early hypo- and late hyperglycaemia in patients with insulin-treated diabetes. Thus, poor glycaemic control may both cause delayed gastric emptying and be a consequence of it.

1.4 GASTROINTESTINAL SYMPTOMS AND DIABETES

In well-performed, controlled studies, the prevalence of GI symptoms is increased in adults with long-standing T1DM [75,76]. Schvarcz et al report that mainly symptoms from the upper GI tract, such as nausea, vomiting, an uncomfortable feeling of postprandial fullness, reflux episodes, and early satiety, but also a feeling of incomplete defaecation, loss of appetite, and abdominal distension, are more prevalent in patients [75]. Mjornheim et al report that moderate to severe symptoms of heartburn or regurgitation, dysphagia, early satiety, nausea, bloating, rectal flatus, constipation, and diarrhoea are more common in adults with T1DM compared with matched controls [76].

The aetiology of GI symptoms in patients with T1DM is probably multifactorial. Transient, as well as chronic, dysmotility of different parts of the GI tract and concomitant diseases, such as coeliac disease and psychiatric disorders, are possible causes of GI symptoms in these patients. Acute hypo- and hyperglycaemia can elicit transient, reversible changes of the motility in several parts of the gut, and long-standing hyperglycaemia can cause permanent changes in the complex neurological and hormonal system regulating gut motility leading to chronic, irreversible dysmotility, as outlined above. However, the relationship between symptoms and gastric emptying in adults is weak [59,63]. But children with T1DM and delayed gastric emptying report dyspeptic symptoms more often than children with T1DM and normal gastric emptying [73]. On the other hand, adolescents with T1DM and chronic dyspepsia have similar gastric emptying rate as non-diabetic adolescents with the same GI symptoms [77].

Another reason for increased prevalence of GI symptoms in patients with T1DM may be that hyperglycaemia per se increases the sensations from the gut through increased cortical response to distension of it [78,79].
Coeliac disease is more prevalent in patients with T1DM than in healthy individuals [80]. All paediatric patients with T1DM in Sweden are screened for presence of coeliac-specific antibodies at diagnosis and thereafter regularly. Symptoms of untreated coeliac disease depend on the age of presentation. Children may experience poor weight gain, failure to thrive, diarrhea, and abdominal pain. Other symptoms include constipation and bloating, and adults may suffer from infertility. However, coeliac disease may also be asymptomatic [80]. Whether diet-treated coeliac disease is associated with GI symptoms is not known, but there is some concern that patients with coeliac disease may have an insufficient intake of dietary fibres, which may cause constipation.

High proportions of both adolescents and adults with T1DM have psychiatric disorders [81,82], and psychological distress is related to GI symptoms in patients with diabetes [83]. Thus, patients with T1DM may have more GI symptoms than healthy people secondary to impaired psychological well-being.

GI symptoms may also be associated with dietary intake. 10–11-year-old-children with GI symptoms of functional origin have poorer food habits than other children [84]. Whether the dietary education given to patients with T1DM has any impact on GI symptoms is not known.

The prevalence of GI symptoms in adolescents with T1DM is poorly investigated. Vazeou et al report that such symptoms are not more common than in healthy controls [85]. However, their patients were not recruited from a population-based setting and the control subjects did not come from the general population, and therefore their results may not be fully reliable.

1.5 GASTRIC EMPTYING, POSTPRANDIAL GLYCAEMIA AND DIABETES

After meal ingestion, the postprandial motor pattern starts which is an irregular activity that lasts for one to two hours [37]. The gastric content is processed mechanically to small fragments making the ingested food fluid before leaving the stomach. The “ileal brake” inhibits too rapid emptying of calories into the duodenum and is activated by the presence of unabsorbed nutrients in the ileum. Approximately 200 kcal per hour is emptied into the duodenum [86]. The distribution of energy-providing nutrients has less importance on gastric emptying rate than the total energy content of the meal. GLP-1 and peptide YY are most likely the two hormones responsible for mediating the “ileal brake” effect [87].

Several hormones inhibit gastric emptying in humans, namely peptide YY, neuropeptide Y, GLP-1, CCK, and orexin A [43,46,47,49]. On the other hand, motilin and ghrelin increase the gastric emptying rate [45,88].

The gastric emptying rate is a major determinant of the postprandial glycaemic level in adults with and without diabetes [89-91]. It seems like much of the observed variation in glycaemic response to different foods, the glycaemic index, is secondary to differences in gastric emptying rates [92]. Addition of fat to a carbohydrate meal delays
gastric emptying and reduces the postprandial glycaemic response in healthy individuals and in adults with T2DM [93,94], but that effect has not been investigated in patients with T1DM. Addition of vinegar to a mixed meal also delays gastric emptying and reduces the glycaemic response in healthy individuals [95]. Adults with T1DM and gastroparesis require less insulin the first two postprandial hours compared with T1DM patients with normal gastric emptying [96].

The postprandial glucose concentration is important since it affects the overall glycaemic control. In adults with T2DM and HbA1c in the lower pathological range, the postprandial glucose levels contribute more to the elevation of HbA1c than the fasting glucose levels [97]. Furthermore, postprandial hyperglycaemia is an independent risk factor for CVD in patients with T2DM and in adults with isolated post challenge hyperglycaemia [98]. The same is probably true also for patients with T1DM. The mortality rates are considerably higher in patients with T1DM than in the general population in all ages and CVD is the leading cause of death for patients with T1DM dying at the age of 30 years or more [99]. Thus, the effects of different foods and meal compositions on the gastric emptying rate and the postprandial glycaemic response are highly important in patients with T1DM, but the literature on this issue is very sparse.

1.6 THE INCRETIN HORMONES

Already in the early 1900s, the idea of factors produced by the intestinal mucosa capable of stimulating endocrine pancreas and thereby lowering the urinary glucose concentration in diabetic patients was introduced [100]. In the 1960s, it was observed that insulin secretion was augmented after oral glucose intake compared with intravenous (iv) glucose infusion, which was interpreted as a probable stimulatory effect on insulin secretion of a humoral substance released from the intestine during glucose absorption [101,102]. That humoral substance was later named incretin. Thus, an incretin is a substance released from the small intestine in response to an oral intake that stimulates insulin secretion. The incretin effect is now estimated to account for approximately 50–70% of all insulin secreted in response to oral glucose administration and it is glucose-dependent [103].

The first hormone shown to be an incretin in humans was gastric inhibitory polypeptide (GIP), known to inhibit the secretion of gastric acid [103]. When it was observed that GIP only inhibits gastric acid secretion at supraphysiological levels [104] but stimulates insulin secretion at physiological levels, the hormone was renamed glucose-dependent insulino-tropic polypeptide but kept its acronym GIP. The second incretin hormone to be described was GLP-1 [105]. GIP and GLP-1 are so far the only known incretin hormones [103].

1.6.1 GIP

GIP is synthesised within and released from intestinal K-cells [106], which are mainly located in the duodenum and proximal jejunum, but are also found in the entire small intestine. Expression of the GIP gene has also been found in the stomach. Biologically active GIP, also called GIP(1-42) or intact GIP, contains 42 amino acids and is
produced after post-translational processing of the proGIP precursor protein containing 153 amino acids [103].

GIP is released in response to absorption of carbohydrates and fat in the gut, whereas protein does not seem to stimulate GIP secretion [107,108]. The secretion is augmented when the energy intake is increased [109]. Somatostatin appears to inhibit the secretion in a paracrine way [110]. Intact GIP is rapidly degraded to the inactive metabolite GIP(3-42) by the enzyme dipeptidyl peptidase IV (DPP-IV), which cleaves off the two N-terminal amino acids [111]. The half-time for intact GIP is approximately 7 min in healthy humans and 5 min in adults with T2DM [112] and the kidney is the major pathway for clearance of the metabolite [113].

1.6.2 Actions of GIP

The GIP receptor (GIPR) is a 7-transmembrane-spanning, G-protein–coupled receptor and its gene is expressed in the pancreas, stomach, small intestine, adipose tissue, adrenal cortex, pituitary, heart, testis, endothelial cells, bone, trachea, spleen, thymus, lung, kidney, thyroid, and several regions in the CNS [103].

The primary role for GIP is to act as an incretin hormone. After binding to its receptor on the pancreatic β-cells, it initiates a cascade of intracellular activities leading to stimulation of glucose-dependent insulin release. Other actions on the β-cells are enhancement of insulin gene transcription and biosynthesis, increase of glucose sensitivity, and promotion of β-cell proliferation and survival [103]. GIP acts also on the pancreatic α-cells by increasing their glucagon secretion in healthy individuals during euglycaemia, but not during hyperglycaemia [114].

Extra-pancreatic actions include stimulation of lipogenesis [115], which is of interest for this thesis, bone formation [116,117], and progenitor cell proliferation in the hippocampus in the CNS [118], as well as inhibition of bone resorption [119]. It has been hypothesized that GIP signals to different tissues in the body that there is enough of nutrient supply for anabolism.

GIP does not inhibit gastric emptying in humans [120] and is not shown to influence energy-intake or satiety.

1.6.3 GIP and diabetes

Adults with T2DM have normal or increased postprandial GIP concentrations compared with healthy controls [109,121,122]. Adults with T1DM have normal postprandial GIP responses [109]. The elimination rates for intact GIP and its metabolite, respectively, do not differ between obese adults with T2DM and healthy obese controls [123]. Exogenous GIP administration does not improve the secretory capacity of the pancreatic β-cells in patients with T2DM as much as in normal subjects or as much as GLP-1 administration does [124] and GIPR agonist treatment has for that reason not been developed for patients with T2DM. Due to the effects of GIP on the lipid metabolism [115], GIPR antagonist treatment for obesity has been considered, but the impaired postprandial insulin secretion would be disadvantageous for the glucose
metabolism. Therefore, GIPR agonist or antagonist therapy for either T2DM or obesity, respectively, will not be an option, at least not in the near future [125].

1.6.4 GLP-1

GLP-1 is released from intestinal L-cells, which are mainly located in the distal ileum and colon, but like GIP, GLP-1 is produced and secreted from all regions of the human small intestine. The secretion of GLP-1 has an early phase (within 10–15 min) and a later, longer phase (30–60 min). The early-phase release is thought to be mediated through nervous or endocrine stimulation, while the late-phase release is thought to be a consequence of direct stimulation of nutrients on the L-cells [103].

Biologically active truncated GLP-1 molecules, GLP-1(7-37) and GLP-1(7-36)NH₂, are secreted after modification of full length inactive GLP-1(1-37) and GLP-1(1-36)NH₂. The addition of the amide group (NH₂) may increase survival in the circulation. In humans, the majority of circulating GLP-1 is GLP-1(7-36)NH₂. GLP-1 and glucagon are produced after post-translational processing of proglucagon, a 180 amino acid peptide, in intestinal L-cells and pancreatic α-cells, respectively. Other cleavage products from proglucagon are liberated as well, including glicentin and glucagon-like peptide 2 [87,103].

The fasting, low levels of GLP-1 increase significantly after ingestion of carbohydrates, fat, or protein [108]. In healthy adults, the response is increased as energy content of the meal is increased [109]. Somatostatin appears to inhibit the secretion in a paracrine way [110]. Insulin and galanin may also inhibit it [103]. GLP-1 is rapidly degraded by the same enzyme as GIP, DPP-IV, which cleaves off the two N-terminal amino acids. The metabolites GLP-1(9-36)NH₂ or GLP-1(9-37) are then produced. GLP-1(9-36)NH₂ has been shown to be an antagonist of GLP-1(7-36)NH₂ at the GLP-1 receptor (GLP-1R) in vitro, but in vivo effects have not been shown yet. The degradation does not only take place in the circulation, but also before the intact peptide reaches the circulation. The half-time for intact, active GLP-1 is less than two min [87]. The kidney is the major pathway for elimination of the GLP-1 metabolites [113].

1.6.5 Actions of GLP-1

Only one GLP-1R has been found so far, despite numerous efforts to find more receptors. Like the GIPR, the GLP-1R is a 7-transmembrane-spanning G-protein–coupled receptor and it is found in pancreatic islets, the CNS, heart, kidney, lung, pituitary, skin, vagus nerve, and the GI tract including the stomach [87,103].

The biological actions of GLP-1 are several, both peripheral and central. The effects on pancreatic β-cells are similar to those of GIP. After binding to its receptor, GLP-1 initiates a cascade of intracellular activities leading to glucose-dependent insulin secretion [105,126]. GLP-1 promotes insulin gene transcription and biosynthesis and thereby inhibits exhaustion of β-cell reserves [127,128]. GLP-1 restores glucose sensitivity in glucose resistant β-cell reserves [129] and increases β-cell mass by stimulation of β-cell proliferation and neogenesis and by inhibition of apoptosis [130,131].
GLP-1 also influences the α- and the δ-cells in the pancreatic islets, leading to reduced glucagon secretion, which is opposite to the effect of GIP, and increased somatostatin secretion, respectively [124,132,133]. Administration of the GLP-1R antagonist exendin(9-39)NH2 to healthy humans increases the glucagon levels [134], indicating that even the low basal, fasting, endogenous GLP-1 level exerts an inhibitory effect on glucagon secretion. That effect is, like the stimulatory effect on insulin secretion, glucose-dependent.

*In vitro* studies show that GLP-1 promotes glycogenesis in hepatocytes and skeletal muscle, increases glucose uptake in fat and muscle, promotes glucose metabolism in adipocytes and skeletal muscle, and inhibits hepatic glucose production. GLP-1 has both lipolytic and lipogenic actions in adipocytes. However, it is not known whether these effects are secondary to changes in insulin and glucagon levels or a direct effect by activation of the GLP-1R on these tissues [103].

GLP-1 reduces appetite and food intake, and tends to decrease the body weights in healthy adults and in patients with T2DM [135-137]. The effect of GLP-1 on satiety is probably mediated in at least two different ways. GLP-1 is readily diffused across the blood-brain barrier and acts on its receptor in the hypothalamus. GLP-1 also acts via its receptor on vagal afferents. These afferents terminate in the nucleus of tractus solitarius in the brainstem and communicates with the hypothalamus, where appetite, hunger, satiety, and food intake are regulated [103].

Furthermore, GLP-1 inhibits gastric acid secretion, gastric emptying, and pancreatic exocrine secretion [47]. These effects are probably mediated in similar ways as the effect on satiety, but includes probably also the regulation of the efferent parasympathetic outflow from the CNS to the intestine and pancreas [87]. The effect on gastric emptying is of interest for this thesis.

GLP-1 may also have cardiovascular effects. It increases systolic and diastolic blood pressure and heart rate in animals, but not in humans [103]. A GLP-1 infusion improved cardiac function in patients with left ventricular dysfunction after an acute myocardial infarction according to a nonrandomized pilot study [138]. However, it is not known whether that was a direct effect of GLP-1 or an indirect effect due to the improved metabolic state. Glucose-insulin-potassium infusions are beneficial in patients with acute myocardial infarction, but the volume requirements associated with that can have adverse effects in patients with left ventricular dysfunction. That problem is avoided by a GLP-1 infusion.

1.6.6 GLP-1 and diabetes

The widespread actions of GLP-1 on glucose metabolism show that GLP-1 is of significant importance for both fasting and postprandial normoglycaemia and its role in pathogenesis and treatment of diabetes has drawn much attention. Both impaired glucose tolerance and T2DM are associated with diminished postprandial insulin secretion, indicating that the incretin effect may be impaired. This suggestion is supported by the findings of reduced postprandial GLP-1 responses in adults with
T2DM [109,121]. These diminished responses are not due to increased elimination [139].

In contrast to GIP, patients with T2DM are responsive to the incretin effect of exogenous GLP-1 [124]. Exogenous GLP-1 administration improves glycaemia in patients with T2DM [137] and the mechanisms are several. Increased glucose-dependent insulin secretion [124,140-142] and reduced glucagon secretion [141,142] are important effects, as well as increased insulin sensitivity [137]. Improvements of postprandial hyperglycaemia are probably also due to the inhibitory effect of GLP-1 on gastric emptying [143-145]. That effect is dose-dependent, but not glucose-dependent. Since the effects on insulin and glucagon secretion are glucose-dependent, the risk for hypoglycaemia is very low compared with other anti-diabetic treatments, such as insulin and sulfonylureas. The effects of GLP-1 on satiety and food intake may also be beneficial for adults with overweight with and without diabetes.

On the other hand, the postprandial GLP-1 response is normal in adults with T1DM [109]. But also in adult patients with T1DM, beneficial effects of exogenous GLP-1 administration on glycaemia have been found [146]. Fasting hyperglycaemia is improved in adults with T1DM by a pharmacological dose of GLP-1 and that seems to be due to reduced glucagon levels and marginally increased insulin levels [132]. Postprandial hyperglycaemia is also reduced in patients with T1DM by a pharmacological dose of GLP-1 [147], probably by inhibition of the gastric emptying rate. In addition, patients with newly diagnosed T1DM may benefit from GLP-1 treatment due to the stimulatory effects of GLP-1 on β-cell mass. However, no such studies have been performed yet, and no studies of GLP-1 therapy in children and adolescents with T1DM have been published yet.

Due to the rapid degradation of GLP-1, it has been hard to develop a suitable pharmacological agent. GLP-1 analogues with extended biological half-lives have now been developed, as well as DPP-IV inhibitors that increase the activity of endogenous GLP-1 by prolonging its half-time [148]. So one hundred years after the first attempts to treat diabetes with an incretin, it has become a reality, at least, for adults with T2DM [100].

1.7 GHRELIN

Growth hormone-releasing hormone (GHRH) stimulates growth hormone (GH) release from the anterior pituitary, while somatostatin inhibits it. Small synthetic molecules called growth hormone secretagogues (GHSs) were found during the 1970’s and 1980’s to stimulate GH release by a pathway different from that of GHRH, which implied that there would be a third receptor regulating GH release [149]. In 1996, such a receptor was described, a 7-transmembrane-spanning G-protein–coupled receptor located in the pituitary gland and the hypothalamus, and it was called the GHS receptor (GHS-R) [150]. An endogenous ligand for that receptor was described 1999 and given the name ghrelin [151]. Ghrelin is the first hormone known to be orexigenic and it has gained significant scientific attention.
Ghrelin is a peptide hormone of 28 amino acids and the active form is modified by acylation with a fatty acid, n-octanoic acid, in serine at position 3. Octanoylation had not been observed as a post-translational peptide modification until it was found on ghrelin [151]. Ghrelin needs to be acylated to exert actions on the GHS-R type 1a, which is responsible for GH secretion. But other actions of ghrelin are independent of the acylation, indicating that there are other, not yet identified, subtypes of the GHS-R [152].

The ghrelin receptor, i.e. the GHS-R, is similar to the motilin receptor and it seems like motilin can stimulate the ghrelin receptor. The ghrelin receptor mRNA is expressed in the pituitary, in many nuclei of the hypothalamus, and in other parts of the CNS, as well as in many peripheral tissues, such as heart, lung, liver, kidney, pancreas, stomach, intestine, adipose tissue, and immune cells [149].

The precursor of ghrelin, preproghrelin, contains 117 amino acids and shows similarity to the precursor of motilin [153]. Furthermore, ghrelin and motilin have similar structure and gastric functions. Acylated and desacyl ghrelin, des-Gln14-ghrelin, and obestatin are all produced from preproghrelin. Des- Gln14-ghrelin has the same biological potency as acylated ghrelin [149].

Approximately 90% of the total circulating ghrelin is nonacylated, called desacyl ghrelin, and the rest is acylated [154]. Desacyl ghrelin was first thought to be inactive, but recent research has shown that desacyl ghrelin has opposite effects on glucose metabolism, food intake, body weight, and gastric emptying to those of acylated ghrelin. However, desacyl ghrelin do not exhibit any neuroendocrine effects, i.e. influence on pituitary hormone release [152,155].

Ghrelin is mainly produced by the stomach, but also in the pituitary gland, hypothalamus, duodenum, jejunum, ileum, colon, heart, endocrine pancreas, kidney, testis, ovary, thyroid gland, placenta, T-cells, neutrophyl granulocytes, and several tumours [149,153]. The half-life of circulating ghrelin is 30 min, and proteases and tissue esterases inactivate and degrade the peptide [153]. The kidney seems to be important for the clearance of ghrelin [154].

Ghrelin has widespread actions. Some of them have so far only been found using pharmacological doses of exogenous ghrelin and the physiological role for ghrelin in all these actions are not fully known yet. However, ghrelin stimulates the secretion of GH, prolaktin, and adrenocorticotropin from the pituitary and it stimulates gastric motility, gastric acid secretion, appetite, food intake, body weight gain, and fat-mass deposition. Furthermore, ghrelin influences endocrine pancreatic secretion, glucose and lipid metabolism, cell proliferation, and cardiovascular and inflammatory functions [149,152,153,156]. Here I will focus on the effects of acylated ghrelin on appetite, food intake, body weight, glucose metabolism, gastric motility, and the GH/insulin-like growth factor-I (IGF-I) axis. I will also review what is known so far about ghrelin levels in patients with T1DM, particularly in the paediatric population.
1.7.1 Ghrelin, appetite, food intake, and body weight

The circulating levels of ghrelin increase preprandially and decrease within 60 min postprandially [157] and the postprandial suppression in healthy adults is proportional to the quantity of ingested calories [158]. These findings suggest a role for ghrelin in meal initiation or as a signal to stop eating.

Exogenous ghrelin enhances food intake and increases body weight in a dose-dependent way in rats [159] and increases appetite and food intake in healthy adult humans [160]. This appetite-stimulating effect involves both orexigenic and anorexigenic pathways in the arcuate nucleus of the hypothalamus, a site that regulates hunger and satiety. Ghrelin stimulates the activity of neurons expressing neuropeptides Y and agouti-related protein. These substances are orexigenic. Ghrelin may also inhibit anorexigenic neurons, which express pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript. These latter substances inhibit appetite. Ghrelin may also stimulate appetite via the vagus nerve. Like GLP-1, ghrelin can stimulate its receptor on vagal afferents, which terminate in the nucleus of tractus solitarius in the brainstem. That nucleus communicates with the hypothalamus [161].

Ghrelin may also influence long-term regulation of body weight. Ghrelin-null mice have similar size, growth rate, food intake, and body composition as wild-type mice [162], but young ghrelin-null mice do not gain weight by chronic exposure to a high-fat diet. These mice have higher energy expenditure and locomotor activity and lower adiposity than wild-type mice [163]. Exogenous ghrelin given for two weeks to wild-type mice increases their weight gain and adiposity by decreased fat and increased carbohydrate utilization [159]. These findings indicate that ghrelin is lipogenic, which has been shown in \textit{in vitro} studies [161], and promotes adiposity in a diet-dependent way.

Circulating basal ghrelin levels are negatively associated with the body mass index (BMI) in children, adolescents, and adults [164-167]. The levels are decreased in obesity and increased in anorexia nervosa, but tend to be normalized by normalization of BMI [164], indicating that ghrelin is a good marker of nutritional status. The reduced basal levels of ghrelin in obesity may be due to inhibition of ghrelin secretion by leptin or insulin [165] aiming at protecting the individual from further weight gain.

Obese adults with reduced insulin sensitivity have depressed fasting ghrelin levels and absent postprandial ghrelin suppression [168]. That finding is of interest for this thesis. Patients with Prader-Willi syndrome, a disorder characterized by mental retardation and hyperphagia leading to severe obesity, have increased fasting ghrelin levels and reduced postprandial suppression. Thus, ghrelin may be responsible for at least some of the insatiable appetite and obesity seen in patients with this syndrome [161].

1.7.2 Ghrelin, glucose metabolism, and insulin levels

Circulating ghrelin concentrations are often inverse to insulin levels in humans [157,158]. Before meals, ghrelin levels are high and insulin levels low in healthy subjects and postprandially the opposite is found. Insulin is required for normal postprandial suppression of ghrelin in humans [169].
The effect of ghrelin on insulin secretion is somewhat controversial. Both stimulatory and inhibitory effects have been reported [153]. For example, an in vivo study on rats showed that exogenous ghrelin stimulates the insulin secretion [170], while an in vitro study on isolated rat pancreatic islets showed that ghrelin inhibits glucose-dependent insulin release in a paracrine way [171]. In healthy humans, the acute effects of exogenous ghrelin administration in a pharmacological dose include, besides increased GH levels, increased plasma glucose and reduced insulin levels [172]. The hyperglycaemia came before the insulin levels dropped, indicating that ghrelin may have a direct glycogenolytic effect. A three hours infusion of ghrelin to healthy young men increased the circulating concentrations of plasma glucose and other substances, such as free fatty acids and GH, but the insulin levels remained constant until the infusion stopped [173]. After the termination of the infusion, the insulin levels rose and the glucose levels normalized.

On the other hand, chronic administration of a GHS to healthy obese men leads to elevations of both glucose and insulin levels during an oral glucose tolerance test after two weeks treatment [174]. However, this effect may be due to increased GH secretion leading to impaired insulin sensitivity rather than a direct effect of the GHS on insulin secretion. This is supported by the finding that another GHS did not influence glucose and insulin levels, but only GH levels [172], indicating that the effects of ghrelin on glucose and insulin levels are mediated via a different receptor, a subtype that GHSs do not bind to. There is evidence from in vitro studies supporting this assumption [171].

In summary, ghrelin effects insulin secretion and the effect is predominantly inhibitory. However, the long-term effect of physiological ghrelin levels is not known yet.

Exogenous ghrelin increases the plasma glucose levels in humans [172,173], probably both by inhibiting appropriate insulin secretion and by direct effects on hepatic glucose output.

Both glucose and insulin levels may on their part influence ghrelin levels. Oral and iv administration of glucose decreases plasma ghrelin concentrations, as well as an euglycaemic hyperinsulinaemic clamp and an insulin-induced hypoglycaemia [152], indicating that insulin may reduce ghrelin levels. Hyperinsulinaemia is associated with low basal plasma ghrelin values in humans [166,175]. The prevalence of T2DM is increased in people with low plasma ghrelin levels [175] and low ghrelin levels may serve as a biomarker of the metabolic syndrome [176].

1.7.3 Ghrelin and gastric motility

Exogenous ghrelin stimulates gastric interdigestive motility [177] and gastric emptying [88] in healthy adults. Also in patients with gastroparesis, the gastric emptying rate is increased by ghrelin [178,179]. These effects are thought to be mediated by the vagal nerve and the ENS [45]. A high fasting, endogenous ghrelin level is associated with a high gastric emptying rate in lean, healthy adults [180]. However, in obese adults, no association is found between endogenous ghrelin levels and gastric emptying [180].
1.7.4 Ghrelin and the GH/IGF-I axis

Exogenous ghrelin stimulates GH release in a dose-dependent way in humans [181,182], although this effect is thought to play a minor role in the physiological regulation of GH release. On the other hand, GH administration in vivo decreases the expression of ghrelin mRNA in the stomachs of rats [170] and GH treatment to GH deficient adult humans leads to reduced fasting ghrelin levels [183]. Furthermore, patients with acromegaly have reduced fasting ghrelin levels and absent postprandial ghrelin suppression [184]. These findings indicate that GH inhibits ghrelin secretion, which may be of interest for this thesis. However, it is not clear whether GH exerts a direct effect on ghrelin regulation or indirect through increased insulin levels.

Some of the effects of GH may be secondary to concomitant changes in ghrelin secretion. For example, the reduction in body fat mass seen after the initiation of GH treatment to GH deficient adults correlates with the reduction in ghrelin levels, indicating that the change in body composition may have been promoted by reduced ghrelin levels [183]. It is also possible that the reduction in body fat mass and BMI seen in patients with Prader-Willi syndrome treated with GH [185] is in part due to reduced ghrelin levels [186].

IGF-I and II are bound to different binding proteins (IGFBPs) in the circulation. Ghrelin is found to positively associate with IGFBP-1 in children and adolescents with and without T1DM [167,187], which may be secondary to the inhibiting effect of insulin on the secretion of both proteins. A negative correlation between ghrelin and IGF-I in children and adolescents with and without T1DM has also been described [188], but not found in other studies [167,187].

1.7.5 Ghrelin and type 1 diabetes

Fasting ghrelin levels, both acylated and total, are reduced in children and adolescents with T1DM compared with healthy controls [187,189,190]. However, Martos-Moreno et al report reduced levels of acylated ghrelin only at diagnosis (before the initiation of insulin therapy) and not after four months of therapy [190] and Bideci et al do not find any difference in total ghrelin levels between patients with T1DM and controls [188]. Preprandial total ghrelin levels decline significantly between time of diagnosis and three months later in children and adolescents with T1DM [191], indicating that ghrelin levels are reduced by insulin therapy or by improved plasma glucose levels in these patients.

Reduced fasting ghrelin levels in children and adolescents with T1DM may be secondary to their increased BMI, peripheral hyperinsulinaemia, increased insulin resistance, or increased GH levels (see below), but the mechanism is not known yet.

Postprandial ghrelin levels have not been investigated as much as preprandial levels in patients with T1DM. Adults with T1DM given at least basal insulin have normal postprandial ghrelin suppression [169]. Female adolescents and young adults with T1DM have more suppressed ghrelin levels after lunch when they inject a bolus dose of a rapid-acting insulin analogue before both breakfast and lunch compared with a single injection of regular and NPH insulin in the morning [192]. This difference may be due
to the absent increase in circulating insulin concentrations at lunch time found when injecting a single dose of regular and NPH insulin in the morning, and indicates that MDI therapy may be superior to conventional insulin therapy in patients with T1DM also from this perspective.

Adolescents with newly diagnosed T1DM (three and nine months after diagnosis) do not have suppressed ghrelin levels postprandially [193]. However, the lack of suppression may be due to methodological weaknesses in that study. Most importantly, the ghrelin concentration was only analysed in one sample taken postprandially and not repeatedly. On the other hand, it is possible that adolescents with T1DM have poor postprandial suppression since they have reduced basal levels and since absent postprandial suppression is reported in patients with similar features as adolescents with T1DM, i.e. overweight, insulin resistance, increased GH levels (see below) [168,184].

The different actions of GIP, GLP-1, and ghrelin are summarized in Table 1.

1.8 THE GH/IGF-I AXIS IN ADOLESCENTS WITH TYPE 1 DIABETES

The concentrations of sex hormones, GH, and IGF-I increase during normal puberty leading to development of secondary sex characteristics and increased growth velocity. In adolescents with T1DM, the GH levels are increased even more, but the IGF-I levels are lower than in healthy puberty-matched adolescents [194,195]. The reason for this abnormality is thought to be the relative hepatic insulinopenia seen in patients with T1DM [196].

In healthy subjects, the insulin concentration in the portal circulation is high due to the secretion of insulin from the pancreatic β-cells, but in individuals lacking endogenous insulin production insulin is delivered to the subcutis leading to high concentrations in the peripheral circulation and low concentrations in the portal circulation. The hepatic GH receptor is in part insulin-dependent [197] and may, because of the low insulin concentration in the portal circulation, be partly resistant, i.e. have fewer binding sites or an attenuated signaling response, in patients with T1DM. IGF-I is mainly produced in the liver and the production is stimulated by GH. When the hepatic GH receptor is insensitive, less IGF-I is produced and secreted to the circulation, even when the GH levels are increased.

IGF-I exerts a negative feedback effect on GH secretion at the hypothalamic or pituitary level [198] and reduced IGF-I levels will therefore lead to increased GH levels. The pulse amplitude, the baseline concentrations of GH, and slightly also the pulse frequency are all increased in adolescents with T1DM [194,199].

GH increases both hepatic and peripheral insulin resistance leading to increased hepatic glucose production and reduced glucose utilization [200]. The hypersecretion of GH in pubertal patients with T1DM contributes to their dramatic increase in insulin resistance [30], which deteriorates metabolic control [31], leads to a need for higher insulin doses, and therefore a risk for increased weight gain.
Table 1. Summary of actions of GIP, GLP-1, and ghrelin.

<table>
<thead>
<tr>
<th></th>
<th>GIP</th>
<th>GLP-1</th>
<th>Acylated ghrelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite and food intake</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Body weight</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Body fat mass</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Fat utilisation</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Plasma glucose concentration</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Insulin secretion</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glucagon secretion</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>GH secretion</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Gastric emptying rate</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Increase.
- Decrease.
The disturbances of the GH/IGF-I axis in patients with T1DM have also been associated with diabetic microvascular complications [196,201].

IGF-I and II are bound to different high affinity IGF binding proteins (IGFBPs) in the circulation. IGFBP-3, the principal circulating binding protein, is GH dependent and its circulating levels are often lower in adolescents with T1DM compared with puberty-matched controls [202]. IGFBP-1 is a 234 amino acids, non-glycosylated protein with a 100-fold lower serum concentration than IGFBP-3 and thus binds only a small fraction of circulating IGF-I [203]. IGFBP-1, which mainly is produced in the liver and is insulin regulated [204], appears to be an inhibitor of IGF-I bioactivity [205] and its circulating levels are elevated in adolescents with T1DM [202]. This increase in IGFBP-1 levels is thought to reduce IGF-I actions on glucose disposal, either by inhibiting IGF-I stimulated glucose uptake in human skeletal muscle [206] or by inhibiting IGF-I negative feedback on pituitary GH secretion. Thus, the hypersecretion of IGFBP-1 may also deteriorate glycaemic control in adolescents with T1DM.

The circulating concentrations of GH, IGF-I, and IGFBPs are influenced by food intake and energy status in humans. Obese humans, who are hyperinsulinaemic, have reduced GH concentrations [207] and, due to insulin-mediated improvements in GH receptor function, normal IGF-I levels. In contrast, anorexic subjects with low insulin secretion have poor GH receptor function and despite of increased GH levels, they have reduced IGF-I levels [208]. Short-term fasting decreases the IGF-I levels in healthy subjects [209] and an optimal intake of both energy and protein is necessary for rapid restoration of these levels after fasting [203]. A low protein intake reduces the IGF-I response to GH in rats [210]. The total circulating IGF-I levels increase by 19% in healthy 8-year-old boys given a high intake of milk protein (4.0 g/kg and day) during one week, while a similar increase in meat protein intake has no effect on IGF-I levels [211]. A twofold increase in protein intake (from 10 to 20 E%) in isocaloric diets do not increase the IGF-I levels in adults with T1DM [212].

The IGFBP-1 levels fluctuate during the day inversely to the insulin levels, like ghrelin, and are therefore affected by the meal pattern [204,213,214]. Since the circulating concentration of IGFBP-1 is dependent on the portal insulin supply, IGFBP-1 can be regarded as a marker of hepatic insulinization and hepatic insulin sensitivity. Postprandial levels of IGFBP-1 and effects of different meal compositions on IGFBP-1 levels have not been described before in adolescents with T1DM.

### 1.9 EATING DISORDERS IN TYPE 1 DIABETES

ED are classified into three groups: anorexia nervosa (AN), bulimia nervosa (BN), and eating disorder not otherwise specified (EDNOS), where the last group is the most prevalent (60% of all cases) and AN the least prevalent [215]. Binge eating disorder (BED) is currently regarded as part of EDNOS. The primary difference between BN and BED is the lack of a regular use of an inappropriate compensatory behaviour to prevent weight gain in BED.
Approximately three per cent of young women have an ED and probably twice that much have clinically important variants [216]. Two per cent of American adolescent males have disordered eating patterns [217], which include milder eating disturbances than the full-syndrome ED, and 5–15% of cases of AN and BN and 40% of cases of BED occur in males [216]. The prevalence of AN in 1985 was 269.9 per 100,000 for females (i.e. approximately 0.3%) and 22.5 per 100,000 for males (i.e. approximately 0.02%) according to a retrospective American population-based study [218]. The prevalence of BN in adolescents in France is about 1.1% in girls and 0.2% in boys [219].

Some aspects of the dietary management of T1DM and the increased weight found in adolescents with T1DM may trigger disordered eating behaviour in susceptible individuals [11,220]. Thus, T1DM is considered as a risk factor for the development of an ED and as much as 10% of adolescent females with T1DM have an ED [221]. Approximately 50% of adolescent girls with T1DM have disturbed eating behaviour at some point during a five year follow-up period. In 92% of adolescent girls with T1DM and an early disturbed eating behaviour the problem is still present later during a five-year follow-up [220].

Females with an ED and T1DM have higher HbA1c than females without an ED [221] and abnormal eating attitudes in youth and young adults with T1DM are independently associated with the presence of retinopathy [6]. Insulin omission is an effective way to lose weight for patients with T1DM and is associated with EDs [221]. However, insulin omission is dangerous and increases the risk for developing retinopathy and nephropathy [222]. Screening for disturbed eating behaviour should start early in patients with T1DM and should be performed annually according to Colton et al [220].

As for the general population, the research on EDs in patients with T1DM has focused on females. However, the proportion of males seeking medical advice for an ED is increasing and the differences between males and females with an ED in psychopathology and comorbidity are less pronounced than the similarities [217,223]. About 16% of adolescent males with T1DM report unhealthy weight control practices over the past year according to a cross-sectional study [224], although the low response rate in that study makes the result unreliable. Higher total scores on the Eating Attitudes Test-26 in adolescent males with T1DM compared with healthy males have been found [225,226], but that finding may only reflect the patients’ adherence to the diabetic dietary regimen. Self-reported bulimic behaviour is associated with poor glycaemic control in both adolescent females and males with T1DM [225], but insulin omission in order to lose weight is not reported in males as often as in females [224,226,227].

To my best knowledge, there is only one published study on the prevalence of EDs in adolescent males with T1DM in relation to matched controls [226]. That study did not find any case of EDs, but the small sample size limits the power of it. Only 43 boys with and 43 boys without T1DM were included. Moreover, the prevalence of EDs may have increased since that study was published in 1992. Thus, it was of great importance to investigate the prevalence of EDs in adolescent males with T1DM using a larger study sample.
2  HYPOTHESIS AND AIMS

2.1  GENERAL HYPOTHESIS
The dietary intake in adolescents with T1DM influences the glycaemic control, the prevalence of GI symptoms, the gastric emptying rate, as well as the circulating levels of GIP, GLP-1, ghrelin, and IGFBP-1.

2.2  SPECIFIC AIMS
To describe food habits and the energy and nutrient intake in adolescents with T1DM in relation to food habits of controls, current food recommendations, and glycaemic control (I).

To investigate the prevalence of GI symptoms in adolescents with T1DM in comparison with controls and to assess related food habits, socioeconomic and diabetes-specific variables (II).

To investigate the effects of fat supplementation to a meal on postprandial glycaemic response and gastric emptying in adolescents with T1DM, as well as the association between glycaemic response and gastric emptying (III).

To investigate the effects of fat supplementation on postprandial levels of GIP, GLP-1, ghrelin, and IGFBP-1 in adolescents with T1DM, and their associations with glycaemic response and gastric emptying (IV).

To investigate the prevalence of EDs in adolescent males with T1DM compared to healthy controls (V).
3 MATERIALS AND METHODS

The studies I, II, and V are epidemiological, while the studies III and IV are experimental. The epidemiological studies are population-based, cross-sectional, and include matched control groups. The experimental studies have a randomized, cross-over design.

3.1 PATIENTS AND CONTROL SUBJECTS

All patients in these studies are adolescents. There are several reasons for choosing to study this age group. There are dramatic physiological and psychological changes during adolescence, which make T1DM more difficult to treat and cope with. The metabolic control deteriorates during adolescence. For example, the median HbA1c value was 7.2% in patients aged 13–19 years compared with 6.5% in patients aged 0–6 years in 2007 in Sweden [2]. During adolescence overweight develops [5], adherence to dietary recommendations deteriorates [18], and EDs often make their debut. In addition, 63% of all paediatric patients with T1DM in Sweden are 12–18 years old [2], making this age group the largest in the paediatric population, which may imply that this age group is the most relevant to study and easiest to get access to. The duration of diabetes is longer in adolescents than in younger patients and consequently, adolescents are more likely to have developed GI motor disturbances, which may give GI symptoms. Furthermore, adolescents are, in contrast to younger children, autonomous, i.e. they can make their own choice whether or not they are willing to participate in research projects.

All patients in these studies were treated in accord with Swedish guidelines [228] and the nutritional advice given agreed with international recommendations [12,229]. All but one patient in studies I and II and one patient in study V reported that they administered at least three insulin bolus doses daily. The characteristics of participating patients and control subjects in all five studies are shown in Table 2.

In studies I and II, all patients aged 13–19 years living in the counties of Örebro and Värmland, in central Sweden, with T1DM for at least one year were asked to participate. For each of the 196 eligible patients, we found an age- and sex-matched non-diabetic control subject at one of two representative schools in central Örebro. One hundred and seventy-four patients and 160 control subjects agreed to participate (response rate 89% and 82%, respectively). In study II, one of the controls was excluded as she reported having a bowel disease.

All participating patients in study I were asked whether they were willing to participate in the second part of the study (the food recording). Of the 121 patients willing to do that, 60 patients were randomly chosen. Of them, three were excluded by mistake and one was excluded because of concurrent illness during the recording. Of the remaining 56 patients, 38 completed the food recording (response rate 68%).
Table 2. Participating patients and controls. Values are means (SD), if not stated otherwise.

<table>
<thead>
<tr>
<th></th>
<th><strong>Patients</strong></th>
<th><strong>Controls</strong></th>
<th><strong>Patients</strong></th>
<th><strong>Patients</strong></th>
<th><strong>Controls</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Studies I and II</td>
<td>Study I²</td>
<td>Studies III and IV</td>
<td>Study V</td>
<td>Study V</td>
</tr>
<tr>
<td></td>
<td>N = 174</td>
<td>N = 160</td>
<td>N = 7</td>
<td>N = 109</td>
<td>N = 139</td>
</tr>
<tr>
<td>Females vs. males (%)</td>
<td>53 vs. 47</td>
<td>54 vs. 46</td>
<td>71 vs. 29</td>
<td>0 vs. 100</td>
<td>0 vs. 100</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.3 (1.7)</td>
<td>16.3 (1.7)</td>
<td>16.4 (0.7)</td>
<td>16.6 (1.1)</td>
<td>16.4 (1.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6 (11.6)</td>
<td>62.1 (10.9)</td>
<td>70.2 (11.3)</td>
<td>70.8 (12.1)</td>
<td>66.7 (11.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.5 (9.1)</td>
<td>170.4 (8.5)</td>
<td>173.9 (4.7)</td>
<td>177.3 (7.8)</td>
<td>175.6 (7.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 (2.9)</td>
<td>21.3 (3.0)</td>
<td>23.2 (3.4)</td>
<td>22.4 (3.1)</td>
<td>21.6 (3.1)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>7.0 (4.0)</td>
<td>NA</td>
<td>3.7 (1.2)</td>
<td>7.2 (4.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Current HbA1c (%)b</td>
<td>7.9 (1.5)</td>
<td>NA</td>
<td>7.3 (0.7)</td>
<td>7.6 (1.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Daily insulin dose (IU/kg)</td>
<td>1.1 (0.3)</td>
<td>NA</td>
<td>0.8 (0.2)</td>
<td>1.0 (0.3)</td>
<td>NA</td>
</tr>
</tbody>
</table>

² Control subjects in study II were the same as in study I, except for one person being excluded from study II due to reporting having a bowel disease.

b Mono-S standard. NA = not applicable.
The 22 patients fulfilling the inclusion criteria for study I and II but not willing to participate had higher current mean HbA1c (8.6% vs. 7.9%, p = 0.028) than participating patients, but did not differ in any other way. There was no difference between randomly chosen patients for the food recording and patients not chosen, neither between patients completing the food record and patients who did not.

In study V, we identified all males aged 14–18 years with T1DM for at least one year living in the counties of Örebro, Dalarna, Värmland, Södermanland, or Västmanland in central Sweden. Two of the 141 eligible patients were not included as they were not scheduled in time. The control group consisted of age-matched non-diabetic males identified from school records of the same schools as in studies I and II. One hundred and nine patients and 139 control subjects agreed to participate (response rate 78% and 99%, respectively). The 30 non-participating patients had higher mean HbA1c than participating patients (8.5% vs. 7.6% p = 0.007), but did not differ in any other way.

As study V only investigated males, the study region was larger in that study compared with studies I and II, where both sexes were included. We only included patients with duration of diabetes of at least one year in these population-based studies, since the first year after diagnosis often is different from the following years in respect of adherence and adjustment. In contrast, we only included patients with duration of at least two years in studies III and IV, since we did not want to include patients in the remission period in those studies. This was also avoided by choosing patients with good to average metabolic control (HbA1c 6.0–7.5% during the last six months) and a total daily insulin dose of 0.7–1.2 IU/kg. The other inclusion criteria in studies III and IV were: age 15–18 years, Tanner stadium 3–5, no smoking, no other medication (including oral contraceptives) than insulin, treatment with insulin glargine in the afternoon or evening and rapid-acting insulin analogue to every meal and snack. The exclusion criteria were coeliac or any other known GI disease, any significant GI symptom, prior abdominal surgery except appendicectomy, any diabetic complication, on-going significant infection, intake of alcohol the day before examination, intense physical activity the day before examination, and significant hypoglycaemia the night before examination.

The studies III and IV may be regarded as part of the same study. They have the same study population and design, but investigate different aspects of fat supplementation to a meal. In contrast to studies I, II, and V, we only investigated a small number of patients in studies III and IV, a number found to be suitable according to a power analysis performed prior to the inclusion of patients. In addition, studies III and IV do not include any healthy controls, since the purpose of the studies was not to compare the findings in adolescents with T1DM with those in healthy controls, but to investigate a certain phenomenon in our patient group.

3.2 STUDY PROCEDURES

In studies I, II, and V, the patients filled out questionnaires at an ordinary visit to their out-patient diabetes clinic and a blood sample for the analysis of HbA1c was taken. The control subjects filled out the same questionnaires at the school nurse reception. Weight
and height were measured and information on socioeconomic variables and presence of any disease and medication was also obtained from both controls and patients.

The questionnaire used in studies I and II contained a food frequency questionnaire (FFQ), a questionnaire concerning GI symptoms, and questions on socioeconomic and diabetes-related issues, altogether 87 questions. The questionnaire used in study V was Eating Disorder Inventory for Children (EDI-C) and eight additional questions concerning the treatment of diabetes.

Randomly chosen patients in study I also completed a prospective, estimated 4-day food record. Patients and control subjects in study V scoring ≥14 on the “Drive for Thinness” subscale in EDI-C and patients reporting insulin omission to lose weight were also interviewed in a semi-structured way. The cut-off of 14 was chosen in agreement with recommendations in the manual of the instrument [230] and corresponds to the 94th percentile in the original norm sample of women. No equivalent cut-off for males exists. This cut-off yields a high specificity at the expense of less sensitivity. The “Drive for Thinness” subscale is considered to be the single most useful subscale for detection of EDs and is recommended for this use.

The project investigating effects of fat supplementation to a meal (studies III and IV) was performed at the out-patient diabetes clinic at Astrid Lindgren Children’s Hospital in Stockholm. The patients came at 8.00 PM after an over-night fast and had not taken any insulin since the evening before. All patients received two iv cannulas. In a randomized order, they ingested a low and a high-fat meal on different days separated by 6–14 days. The meals were prepared in advance by two dieticians and stored at -20°C.

The meals consisted of pasta with a sauce of tomatoes and ham with or without rape oil. The total energy content was 320 and 640 kcal, and the fat content was 20 kcal (2 grams, 6 E%) and 340 kcal (38 grams, 53 E%) in the low and high-fat meals, respectively. Both types of meals contained the same quantity of carbohydrates (240 kcal, 60 grams) and protein (60 kcal, 15 grams). We chose a pasta meal because such a meal had been used before in patients with T1DM together with the paracetamol absorption method [231], and found to be reliable. Considering the recommended distribution of energy-providing nutrients, the compositions of our test meals were rather extreme. However, we wanted to detect a difference between the meals in the variables investigated without having to include too many patients and therefore, we chose to use test meals with a large difference in fat content.

The subjects were allowed to drink 100 ml of water together with the meal, which was ingested in 15 min in a sitting position. All patients remained in the sitting position during the whole study period. Paracetamol (30 mg/kg, Alvedon® tablet, AstraZeneca Sverige AB, Södertälje, Sweden) was pulverized in a mortar and carefully mixed into the meals as gastric emptying was estimated using the paracetamol absorption method [232-235].

The patients needed to be normoglycaemic (p-glucose 4.0–7.5 mmol/l) when meal ingestion began, i.e. at baseline, to enable accurate measurement of gastric emptying.
Patients with hypoglycaemia (p-glucose < 4.0 mmol/l) at arrival to the diabetes clinic were not investigated that day. Patients with hyperglycaemia (p-glucose > 7.5 mmol/l) were given a variable iv insulin infusion (human insulin 0.02–0.2 IU kg\(^{-1}\) h\(^{-1}\)) prior to investigation to reach normoglycaemia. Before the low-fat meal, six of the seven patients received an insulin infusion, and to them 3.6 IU (2.5–17.3) was given during 42.5 min (30–110). Before the high-fat meal, four of the patients received an insulin infusion, and to them 4.0 IU (0.3–5.8) was given during 37.5 min (5–85). After the end of an infusion at least 30 min passed until baseline. An alternative to this procedure would have been an overnight euglycaemic clamp, which probably would have been preferred. However, it was considered too costly.

At baseline, all patients were normoglycaemic and given the same sc prandial insulin dose (7 IU insulin aspart) to both test meals in the same place (laterally to the umbilicus) on both occasions. Blood samples were taken before and after iv insulin infusion, if given, at baseline, and postprandially repeatedly for four hours. The concentrations of paracetamol, glucose, GIP, GLP-1, ghrelin, and IGFBP-1 were analysed in the blood samples. One of the two iv cannulas was warmed by heating pads for arterilization of the blood and used for analyses of plasma glucose [236].

### 3.3 FOOD FREQUENCY QUESTIONNAIRE AND FOOD RECORDS

The FFQ used in study I was based on a reliable questionnaire previously used in Swedish adolescents [237]. It was slightly amended to fit the needs of persons with diabetes. The first questions dealt with the frequency (from never to four times a day) of eating 34 different articles of food, such as fat, cheese, and meat. Further questions concerned drinks taken together with different meals, percentage of fat in dairy products, type of fat used for home cooking, type of bread, consumption of alcohol, and meal pattern. The FFQ contained altogether 58 questions.

Patients completing the food record entered prospectively on four preset days, three weekdays and one weekend day, all food and drink they consumed in a food diary. To estimate the quantity of ingested foods and drinks, they used ordinary kitchen measures and a portion-guide [238]. The food records were analysed by a dietician using a Swedish software program [239].

There are several methods for assessing dietary intake. A FFQ assesses the usual intake and is useful when large samples are investigated because it is inexpensive. But FFQ can only at best give semi-quantitative information and the list of specific foods may not suit all ethnic cultures. In addition, the validity of a FFQ is uncertain. Direct observation and weighed food records give valid quantitative information on current energy and nutrient intake, but both methods are usually too expensive to use in epidemiologic research and they do not reflect usual intake. These methods may also cause behavioural changes in the subjects being studied. A food history by interview gives quantitative information on recent and usual intake, but is very dependent on the quality of the interviewer, is time-consuming and, therefore, expensive. An estimated food record or diary brings also quantitative information and is often used as the gold standard for validating other methods. However, underreporting is common since it is
tedious to write down everything one eats. There is also a risk for a change in food intake due to the recording. The method is time-consuming and expensive. A 24 hours recall gives quantitative information and is useful especially when the investigation is performed by a well-trained dietician. It depends only on short-term memory and is therefore thought to be more accurate than methods estimating usual intake. However, the information on food intake during a single day is seldom representative. Three to seven recalls may be needed to accurately estimate usual intake [240].

3.4 QUESTIONNAIRE ON GASTROINTESTINAL SYMPTOMS

The questionnaire on GI symptoms used in study II contained 13 questions and originated from a postal, reliable questionnaire previously validated in a Swedish population [241]. It has been used in adults with and without T1DM [75]. The patients and control subjects were asked to answer Yes or No to questions on whether they had been troubled during the last three months by different symptoms. Most of the questions dealt with symptoms from the upper GI tract, since especially these symptoms were more prevalent in adults with T1DM [75]. The last question was open making it possible for patients and controls to describe by their own words any GI symptom not covered by the other questions.

There is no gold standard method for estimating GI symptoms. A questionnaire [242] in English and French on GI symptoms in children and adolescents based on the paediatric Rome II criteria for functional GI disorders in infants, children and adolescents [243] has been developed and partly validated. But that questionnaire is not yet translated into the Swedish language and culture, nor validated in a Swedish adolescent population.

The questionnaire used in study II is easy to fill in and to analyse, but is retrospective, relying on the subjects’ memories. However, this disadvantage is the same for patients as for controls, why it will not yield a differential bias, which is important when interpreting the results. The questionnaire does not take severity, duration, or cause of the symptoms into account. Severity of symptoms may have importance as Mjornheim et al found larger differences between adults with T1DM and controls when moderate and severe symptoms were analysed separately [76].

3.5 ASSESSMENT OF EATING DISORDERS

The EDI-C used in study V was based on the Eating Disorder Inventory (EDI) [244]. The patients and control subjects decided on 91 statements how much they agreed with them by ticking the appropriate box. For each statement, there were six boxes to choose among, ranging from “always” through “sometimes“ to “never”. The statements covered 11 groups, called subscales. Two of these subscales directly concerned EDs, namely “Drive for Thinness” and “Bulimia”, while the other subscales concerned psychopathological factors associated with EDs. These subscales were called “Body Dissatisfaction”, “Ineffectiveness”, “Perfectionism”, “Interpersonal Distrust”, “Interceptive Awareness”, “Maturity Fears”, “Asceticism”, “Impulse Regulation”, and “Social Insecurity”. 
The semi-structured interview used in study V was a teenager-adjusted version of Rating of Anorexia and Bulimia [245], which was based on the Eating Disorder Examination [246] but developed further for clinical use and rephrased for Swedish circumstances. The interview was performed by a child and adolescent psychiatrist well experienced in EDs to determine whether or not an ED according to the criteria in Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) existed, and if so, of what type.

The EDI is a well-established self-administered questionnaire shown to be a reliable and valid screening instrument for EDs in both diabetic and non-diabetic populations [221,230,244]. In contrast to other instruments, EDI is designed to assess psychological characteristics relevant to AN and BN [244]. However, there are other questionnaires for the assessment of EDs. The Eating Attitude Test-26 measures disturbed eating behaviours and consists of three subscales: dieting, bulimia, and oral control [247]. The Bulimic Investigatory Test, Edinburgh, only assesses binge-eating [248].

The use of a two-step procedure, as in study V, is effective and accurate in finding and diagnosing EDs. The questionnaire screens the whole population and subjects at highest risk for having an ED is chosen for the next part of the study, the interview, which validates the results of the questionnaire and gives an ED diagnosis, if appropriate. An ED cannot be diagnosed without an interview performed by a psychiatrist or psychologist.

There are several forms of semi-structured interviews for assessing EDs, for example the Clinical Eating Disorders Rating Instrument [249,250], the Eating Disorder Examination [246,251], and the Structured Interview for Anorexia and Bulimia Nervosa [252,253]. However, the interview used in study V is easy to use, able to discriminate between different eating disorder diagnoses, measures concomitant psychopathology and background variables relevant to treatment planning, is suitable for both clinical work and research in the field of EDs, and is adjusted to Swedish circumstances [245].

3.6 ESTIMATION OF GASTRIC EMPTYING

The gastric emptying was assessed by the paracetamol absorption method [232-235]. This method is based on the following findings: the absorption of paracetamol in the stomach is negligible and the absorption in the small intestine is very rapid. Thus, the absorption of paracetamol is determined by the gastric emptying rate. Serum concentrations of paracetamol correlate with the gastric emptying of both liquids and semi-solids [232,233] and the paracetamol absorption method correlates well with scintigraphic emptying of both liquid and semi-solid meals [234,235]. The method has been used previously to assess gastric emptying of solids in patients with Type 1 DM and found to be reliable [231]. Algorithms taking individual pharmacokinetics of paracetamol into account and transforming paracetamol concentrations into gastric emptying half-time and other emptying parameters have been developed and validated [254,255].
There are several methods for assessing gastric emptying rate. The scintigraphic technique uses a non-absorbable gamma-emitting radionuclide and is often considered as the gold standard. This technique can measure simultaneously the gastric emptying of both liquid and solid components of the meal and also the intragastric meal distribution [90]. Other commonly used methods are ultrasound, magnetic resonance imaging, and marker dilution and aspiration techniques. Another tracer method, than the paracetamol absorption method, uses carbon-labelled octanoic acid as the tracer marker (breath test). Tracer methods rely on the intestinal absorption of the tracer marker [256,257].

The paracetamol absorption method is inexpensive, easy to use, and not dependent on specific equipment, a specially trained person, the administration of a radioactive isotope, or the use of a nasogastric tube. However, the method is not fully standardized, is time consuming, and gives only an indirect estimation of gastric emptying.

3.7 LABORATORY ANALYSES

The Mono-S standard for HbA1c was used in all studies [258]. HbA1c, standardized according to the National Glycohaemoglobin Standardization Program, equals 0.92 times HbA1c measured with the Mono-S standard plus 1.33. In studies I, II, and V, HbA1c was analysed using high-performance liquid chromatography (HPLC; Bio-Rad Laboratories, Hercules, CA, USA) and in studies III and IV, an immunochemical method (Cobas Integra 400, Roche Diagnostics Scandinavia AB, Bromma, Sweden) was used. The reference interval for the HPLC method was 3.5 – 5.3 % and the intra- and inter-assay coefficients of variation (CV) were 1.5 and 2.7 %, respectively. The reference interval for the immunochemical method was < 5.2 % and the intra- and inter-assay CV were < 3 %.

The plasma glucose concentrations in studies III and IV were measured using bedside equipment based on a glucose dehydrogenase method (HemoCue B-Glucose Analyzer, HemoCue AB, Ängelholm, Sweden) during the insulin infusion to manage the infusion rate. The baseline and postprandial plasma glucose concentrations were measured using a glucose oxidase-based method (Synchron LX20, Beckman Coulter AB, Bromma, Sweden) with intra- and inter-assay CV < 4 %.

The serum paracetamol concentrations were measured using fluorescence polarization immunoassay technology (TDx/TDxFLx, Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA). The detection limit was 1.00 µg/ml (6.6 µmol/l) and the intra- and inter-assay CV were < 5 %.

The incretin hormones, ghrelin, and IGFBP-1 concentrations were measured using radioimmunoassays (RIA’s). The total GIP immunoreactivity was measured using the C-terminally directed antiserum R65 [259,260], which reacted fully with intact GIP (GIP(1-42)) and the N-terminally truncated metabolite, GIP(3-42). The assay had a detection limit of 3 pmol/l and an intra-assay CV of approximately 6 %. GIP concentrations below the detection limit were set as 3 pmol/l in the statistical analyses.
The total GLP-1 immunoreactivity was measured as described previously [261], using standards of synthetic GLP-1(7-36)amide and the C-terminally directed antiserum no. 89390, which is specific for amidated GLP-1. The assay cross-reacted < 0.01% with C-terminally truncated fragments and 83% with the N-terminally truncated metabolite GLP-1(9-36)amide, and had a detection limit of 1 pmol/l. Intra- and inter-assay CV were < 6% and 15%, respectively, at 40 pmol/l. GLP-1 concentrations below the detection limit were set as 1 pmol/l in the statistical analyses.

The total ghrelin serum levels were analyzed using a RIA kit from Linco Research (GHRT-89HK; Linco Research Inc., Missouri, USA) according to the manufacturer’s instructions. Intra- and inter-assay CV were 3.3–10.0% and 14.7–17.8%, respectively. The detection limit was 100 pg/ml.

The total IGFBP-1 serum levels were determined by a RIA as described previously [262] and modified from [263]. The detection limit was 3 ng/ml and intra- and inter-assay CV were 5.6% and 11.8%, respectively. IGFBP-1 concentrations below the detection limit were set as 3 ng/ml in the statistical analyses.

The total concentrations of GIP and GLP-1 include both biologically active hormones and their inactive metabolites. As both GIP and GLP-1 are degraded rapidly most of the total concentrations are the inactive metabolites [264]. Newer assays have been developed that only measure the intact hormones [112,265]. Assays for total concentrations use an antibody against the C-terminal, while assays for intact peptides use an antibody against the N-terminal. The N-terminally directed assays have mainly been used for the study of hormone turnover and clearance. The concentrations of total GIP and GLP-1 reflect the secretion of the hormones, while the concentrations of intact hormones reflect their bioactivity.

The total ghrelin concentration includes acylated and desacyl ghrelin. It is measured using a RIA with antibodies against the C-terminal fragments (amino acid positions 13 to 28). For the measurement of only acylated ghrelin, a RIA with antibodies against the N-terminal fragment (amino acid positions 1 to 11 with n-octanoylation at Ser 3) has to be used [266]. Since desacyl ghrelin has some effects that are opposite to those of acylated ghrelin [155], it seems advantageous to measure both total and acylated ghrelin in studies investigating actions of ghrelin.

3.8 STATISTICAL ANALYSES AND ETHICS

All statistical analyses were performed in the software programme Statistical Package for the Social Sciences version 12.0 (SAS Institute, Cary, NC, USA) except for the investigation of associations between glucose and paracetamol concentrations in study III, which was computed as the mixed model and implemented in the procedure MIXED in SAS version 9.1 (SAS Institute, Cary, NC, USA). Statistical significance was set at \( p < 0.05 \).
Results were given as mean ± SD if normally distributed, otherwise as median (range). The area under the curve (AUC) was calculated according to the trapezium rule. The frequency of consuming separate foods and scores on separate subscales of the EDI-C were compared between patients and controls using the Mann-Whitney $U$-test in study I and V, respectively. Comparisons were also made by one sample $t$-test, independent samples $t$-test, and paired $t$-test, where suitable. Wilcoxon test was used for nonparametric, paired comparisons. Correlations were analysed by Pearson’s and Spearman’s Correlation tests, where appropriate, and multiple linear regression models were also used. Proportions were analysed in cross tables and compared by Pearson $\chi^2$ test or Fisher’s exact test, where appropriate. To investigate relationships between glucose and paracetamol concentrations in study III, we used ANOVA for repeated measurements with differences in glucose concentrations between measurements at 90 min and baseline and at 240 min and 90 min as the outcome. Three explanatory variables were used in the model: (1) differences in paracetamol concentrations between the same time points, (2) time points (90–0 min and 240–90 min), and (3) type of meal.

The ghrelin concentrations found did not follow a normal distribution and there was a large interindividual variation as previously reported [187]. Therefore, ghrelin was also expressed relative to the average postprandial concentration for each subject, which made the values normally distributed.

All studies were approved by the local Ethic’s Committees and conducted in accord with the Declaration of Helsinki. All participating subjects gave informed consent, and in studies III and IV, one of the patients’ parents also gave informed written consent.
4 RESULTS

4.1 DIETARY INTAKE (I)

4.1.1 Food habits

Adolescents with T1DM were heavier than controls (p = 0.001 for girls and p = 0.002 for boys). The educational level was higher in parents of controls than in parents of patients (p < 0.001).

Patients consumed sour milk/yoghurt (p = 0.001), peas, beans, and broccoli (p = 0.019), porridge (p = 0.031), fruit and fruit juice (p = 0.006), potatoes and roots (p < 0.001), meat, fish, egg, and offal (p < 0.001) and sugar-free sweets (p < 0.001) more often than controls did. Controls consumed ordinary sweets (p < 0.001) and snacks (p = 0.020) more often than patients did. Eighteen per cent of controls drank sugary soft drink or juice at least once daily, compared to three per cent of patients (p < 0.001).

Patients ate more bread (p = 0.003) and chose coarse rye bread more often and white bread less often than controls. Low-fat butter was used by 81% of the patients and 71% of the controls (ns) and patients drank low-fat milk more often than controls (p = 0.001). In the homes of patients, cooking oil or liquid margarine was most often used for cooking, while in the homes of controls, solid butter or solid margarine was used (p < 0.001). The differences between patients and controls in consumption frequencies were not influenced by BMI SDS, parents’ educational level or origin, except for sour milk/yoghurt.

Patients with coeliac disease (N = 12) consumed bread (p = 0.012), egg (p = 0.045), and ordinary buns, cakes, and biscuits (p = 0.034) less often and potatoes more often (p = 0.025) than patients without coeliac disease. Patients with coeliac disease ate snack in the morning more often than patients without coeliac disease (3.5 times/week vs 2.1 times/week; p = 0.046).

Patients had breakfast (p = 0.001), morning snack (p < 0.001), dinner (p < 0.001), and evening snack (p = 0.032) more often than controls. About 70% of both patients and controls ate the free hot school lunch every school day.

4.1.2 Energy and nutrient intake

The daily energy intake was 8.1 ± 2.8 MJ and 10.2 ± 2.8 MJ for girls and boys, respectively. The intake of energy-providing nutrients is shown in Table 3 together with the Swedish nutritional recommendations (SNR) [267] and diabetes-specific recommendations (ISPAD) [229]. The intake of carbohydrates, sucrose, and total fat followed the international recommendations. The intake of protein was higher than recommended in boys (p = 0.040), but not significantly in girls. In both boys and girls, the intake of saturated fat was higher than recommended (p = 0.004 and p < 0.001, respectively) and the intake of polyunsaturated fat was lower than the SNR (p = 0.008 and p = 0.007, respectively). In girls, the intake of fibre was lower than the earlier calculated recommendation from ISPAD (p = 0.023). The intake of fibre related to the
Table 3. Daily intake of energy-providing nutrients in adolescents with T1DM together with dietary recommendations. Values are means (SD).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Boys</th>
<th>Girls</th>
<th>SNR(^a)</th>
<th>ISPAD(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (E%)</td>
<td>52.8 (8.5)</td>
<td>53.6 (6.0)</td>
<td>55–60</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Sucrose (E%)</td>
<td>6.8 (5.0)</td>
<td>8.5 (4.0)</td>
<td>&lt; 10</td>
<td></td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>16.4 (2.6)</td>
<td>15.8 (2.7)</td>
<td>10–15</td>
<td>10–15</td>
</tr>
<tr>
<td>Fat (E%)</td>
<td>31.1 (7.4)</td>
<td>30.2 (5.5)</td>
<td>&lt; 30</td>
<td>30–35</td>
</tr>
<tr>
<td>Saturated fat (E%)</td>
<td>13.1 (3.6)</td>
<td>13.2 (3.0)</td>
<td>≤ 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Monounsaturated fat (E%)</td>
<td>11.5 (3.0)</td>
<td>10.6 (2.4)</td>
<td>10–15</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Polyunsaturated fat (E%)</td>
<td>4.1 (1.2)</td>
<td>4.2 (1.3)</td>
<td>5–10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>21.5 (10.1)</td>
<td>17.3 (6.9)</td>
<td>25–35(^c)</td>
<td>21(^d)</td>
</tr>
<tr>
<td>Fibre (g/MJ)</td>
<td>2.2 (0.9)</td>
<td>2.2 (0.5)</td>
<td>2.8–3.4(^e)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Swedish Nutritional Recommendations [267]. \(^b\) International Society for Pediatric and Adolescent Diabetes [229]. \(^c\) Recommendation for adults. \(^d\) Calculated recommendation for the age group studied. \(^e\) Newer recommendation [12].

Total energy intake was lower than the newer international recommendation (comparing the mean value of the whole study group with 3 using one sample t-test, \(p < 0.001\)).

The energy distribution on different meals did not differ significantly from the SNR [267] for either girls or boys, although snacks contributed to as much as 30% of the total daily energy intake for girls.
4.1.3 Dietary intake and metabolic control

Patients answering the FFQ and having HbA1c < 7.0% (N = 45, 22 girls) ate peas, beans, and broccoli (p = 0.018), fruit and berries (p = 0.029), and fish (p = 0.021) more often and drank sugar-free juice and soft drinks (p = 0.047) less often than patients having HbA1c ≥ 8.5% (N = 48, 24 girls). Patients fulfilling the food record and having HbA1c < 7.0% (N = 11, five girls) consumed less fat (28 E% vs. 34 E%, p = 0.011) and more carbohydrates (56 E% vs. 49 E%, p = 0.039) than patients fulfilling the food record and having HbA1c ≥ 8.5% (N = 6, four girls).

4.2 GASTROINTESTINAL SYMPTOMS (II)

Both patients and controls reported that they had been troubled during the last three months by on average two GI symptoms each. Seventy seven per cent of both patients and controls reported at least one symptom. The proportions of patients and controls reporting individual symptoms did not differ (see Table 2 in the manuscript “Gastrointestinal symptoms in adolescents with type 1 diabetes”).

More girls than boys reported GI symptoms, in both patient and control groups (patients: abdominal pain (p = 0.013), an uncomfortable feeling of fullness at or after meals (p = 0.003), nausea (p < 0.001); controls: abdominal pain (p = 0.024), early satiety (p = 0.010), nausea (p = 0.005)).

Patients smoking cigarettes daily (N = 10) had more often poor appetite (p = 0.024), loss of weight (p = 0.039), an uncomfortable feeling of fullness at or after meals (p = 0.004), swallowing difficulties (p = 0.005), belching (p = 0.026), and nausea (p = 0.016) than patients not smoking daily. Controls smoking daily (N = 13) reported more often vomiting (p = 0.007) than controls not smoking daily.

4.2.1 Gastrointestinal symptoms and food habits

Early satiety was more prevalent (p = 0.025), and belching (p = 0.057) and swallowing difficulties (p = 0.071) tended to be more prevalent in patients not eating breakfast, lunch, and dinner every day (N = 75) compared with patients eating these meals every day. Controls not eating breakfast, lunch, and dinner every day (N = 100) more often reported abdominal pain (p = 0.013), nausea (p = 0.049), and diarrhoea (p = 0.023) than other controls.

Patients reporting at least one GI symptom drank milk (p = 0.011), ate potatoes (p = 0.002), and meat on sandwiches (p = 0.043) less often than patients without symptoms. Controls reporting at least one GI symptom ate fish less often than controls without symptoms (p = 0.003).

4.2.2 Gastrointestinal symptoms and diabetes-specific variables

Patients who had had diabetes for more than seven years (N = 91) reported more often reflux episodes (p = 0.046) and vomiting (p = 0.006) than patients with shorter
duration. Loss of weight ($p = 0.050$) and reflux episodes ($p = 0.011$) were more prevalent in patients with mean HbA1c during the last year $\geq 8.5\%$ (N = 44) compared with patients with mean HbA1c during the last year $< 7.0\%$ (N = 44).

The prevalence of individual symptoms did not differ between patients with a microvascular complication (N = 17) and patients without, between patients who had experienced ketoacidosis last year (N = 8) and patients who had not, or between patients who had experienced a severe hypoglycaemic episode last year (N = 22) and patients who had not. The type of prandial insulin (rapid-acting insulin analogue or human insulin) did not differ in patients with and without individual GI symptoms. The prevalence of individual symptoms did not differ between patients using an insulin pump (N = 28) and patients using MDI.

Patients with coeliac disease (N = 12) reported more often constipation ($p = 0.030$) than patients without coeliac disease. Otherwise, there was no difference in prevalence of individual GI symptoms between patients with and without coeliac disease.

4.3 POSTPRANDIAL HORMONAL RESPONSES TO AND GASTRIC EMPTYING OF A HIGH AND A LOW-FAT MEAL (III AND IV)

All patients tolerated the study design well, even though many of them disliked the bitter taste of pulverized paracetamol mixed into the meals. One patient developed prolonged, asymptomatic postprandial hypoglycaemia after both meals and another patient only participated once. These two boys were therefore excluded from further analyses.

Each subject’s postprandial glycaemic response (AUC $0-240\text{ min}$) to one of the test meals was correlated with that of the other test meal ($r = 0.765$, $p = 0.045$).

4.3.1 Glycaemic response

There was no difference in plasma glucose levels between the test meals at baseline. None of the participating patients developed hypoglycaemia. During the first two hours, the AUC for glucose concentrations was larger after the low-fat than after the high-fat meal ($p = 0.047$, Figure 1). Time-to-peak in glucose concentration tended to be delayed after the high-fat compared with the low-fat meal (210 min (120–240) vs. 120 min (50–240), $p = 0.080$).

4.3.2 GIP response

The postprandial total GIP concentrations are shown in Figure 1 in manuscript “Effects of fat supplementation on postprandial GIP, GLP-1, ghrelin, and IGFBP-1 levels in adolescents with type 1 diabetes”. Neither initial (i.e. at baseline or before insulin infusion, if given) nor baseline concentrations differed between the test meals. The concentrations increased significantly after both meals (from 3.0 (3–3) pmol/l to 73.0 (20–99) pmol/l, $p = 0.018$, and from 3.0 (3–7) pmol/l to 18.0 (14–34) pmol/l, $p = 0.018$, for high and low-fat meals, respectively). The postprandial peak value ($C_{\text{max}}$), AUC $0-240\text{ min}$ and AUC $0-120\text{ min}$ were larger after the high-fat compared with the low-fat meal ($p = \ldots$)
Figure 1. Mean plasma glucose concentrations (SD) after a high-fat (black triangles) and a low-fat meal (white circles) in seven adolescents with T1DM given 7 IU insulin aspart sc at the beginning of each meal. The AUC was larger after the low-fat than after the high-fat meal during the first two hours (p = 0.047).

0.004, p = 0.002, and p = 0.002, respectively). Time-to-peak did not differ between meals.

4.3.3 GLP-1 response

The postprandial total GLP-1 concentrations are shown in Figure 2 in manuscript “Effects of fat supplementation on postprandial GIP, GLP-1, ghrelin, and IGFBP-1 levels in adolescents with type 1 diabetes”. Neither initial nor baseline concentrations differed between the meals. The concentrations increased significantly after both meals (from 14.4 ± 4.0 pmol/l to 40.4 ± 11.8 pmol/l, p < 0.001, and from 16.1 ± 6.8 pmol/l to 33.7 ± 8.2 pmol/l, p < 0.001, for high and low-fat meals, respectively). The $C_{\text{max}}$ and AUC 0–120 min were larger after the high-fat compared with the low-fat meal (p = 0.023 and p = 0.030, respectively). Time-to-peak tended to be delayed after the low-fat meal (180 (40–210) min vs. 60 (20–240) min, p = 0.075).
4.3.4 Ghrelin response

The postprandial relative ghrelin values are shown in Figure 3 in manuscript “Effects of fat supplementation on postprandial GIP, GLP-1, ghrelin, and IGFBP-1 levels in adolescents with type 1 diabetes”. Neither initial nor baseline absolute ghrelin concentrations differed between the meals. The absolute concentrations decreased significantly after both meals (from 605 (438–1376) pg/ml to 485 (324–1117) pg/ml, p = 0.018, and from 646 (400–1336) pg/ml to 574 (400–1082) pg/ml, p = 0.028, for high and low-fat meals, respectively). The decrease adjusted for baseline value tended to be larger after the high-fat compared with the low-fat meal (17.1 (9.3–34.7)% vs. 13.1 (0.0–30.5)%, p = 0.063). The relative ghrelin values decreased significantly after both meals (p = 0.018 and p = 0.028 for high and low-fat meals, respectively). The AUC 0–240 min for relative ghrelin values was smaller after the high-fat compared with the low-fat meal (p = 0.043). Time-to-reach the nadir did not differ between meals.

4.3.5 IGFBP-1 response

The pre- and postprandial total concentrations of IGFBP-1 are shown in Figure 4 in manuscript “Effects of fat supplementation on postprandial GIP, GLP-1, ghrelin, and IGFBP-1 levels in adolescents with type 1 diabetes”. The initial values, which may at least partly reflect overnight hepatic insulinization, did not differ between the meals. In patients receiving iv insulin infusion (on average 4.0 IU and 3.6 IU before high and low-fat meals, respectively) a rapid and significant decrease in IGFBP-1 concentrations was observed before meal ingestion (p = 0.037 and p = 0.036, for high and low-fat meals, respectively). The concentrations continued to decrease significantly after the standard 7 IU sc dose of insulin given at baseline (p = 0.018 for both meals). The apparent early postprandial rise in IGFBP-1 concentration after the low-fat meal was seen also in some patients after the high-fat meal and both in patients receiving and not receiving iv insulin. The apparent late postprandial rise in IGFBP-1 concentration after the high-fat meal was seen in one patient only. The absolute and the relative decreases from initial value, the AUC 0–120 min, the AUC 0–240 min, and time-to-reach the nadir did not differ significantly between meals.

4.3.6 Gastric emptying

During the first two hours, the AUC for paracetamol concentrations was larger after the low-fat than after the high-fat meal (p = 0.041, Figure 2). Time-to-peak in paracetamol concentration tended to be delayed after the high-fat compared with the low-fat meal (120 min (75–180) vs. 60 min (60–120), p = 0.051).

4.3.7 Glycaemic response and gastric emptying

There were correlations between glucose and paracetamol concentrations at 10, 20, 30, 40, 50, 60, and 75 min after the low-fat meal (r = 0.530–0.788) and at 30, 40, 50, 60, and 120 min after the high-fat meal (r = 0.422–0.728). As analyzed in the mixed model, there was a statistically significant correlation between glucose concentrations at 90 min minus that at 0 min and at 240 min minus that at 90 min and the corresponding differences in paracetamol concentrations (p < 0.001).
Figure 2. Mean serum paracetamol concentrations (SD) after a high-fat (black triangles) and a low-fat meal (white circles) containing 30 mg paracetamol per kg body weight in seven adolescents with T1DM. The AUC was larger after the low-fat than after the high-fat meal during the first two hours (p = 0.041).

4.3.8 GLP-1 response and gastric emptying

A larger early GLP-1 secretion (AUC 0-120 min for GLP-1) was associated with a slower gastric emptying rate (time-to-peak in paracetamol) (r = 0.583, p = 0.029) analysing both meals together. That relationship was not affected by the iv insulin infusion dose as analysed in a multiple linear regression model using time-to-peak in paracetamol as outcome variable. The statistical significance for the correlation between AUC 0-120 min for GLP-1 and time-to-peak in paracetamol was lost when each meal was analysed separately (r = 0.582, p = 0.170 and r = 0.356, p = 0.433 for high and low-fat meals, respectively).

4.3.9 GIP, GLP-1, ghrelin, IGFBP-1, and glycaemia

The postprandial GIP, GLP-1, ghrelin, and IGFBP-1 responses did not correlate significantly with the glycaemic response (AUC 0-120 min for glucose). A higher initial ghrelin level was associated with a lower glycaemic response (p = -0.684, p = 0.007) analysing both meals together, and that association was not affected by the iv insulin
infusion dose as analysed in a multiple linear regression model using AUC \(_{0-120\ min}\) for glucose as outcome variable. The statistical significance for the correlation between initial ghrelin and AUC \(_{0-120\ min}\) for glucose was lost for the high-fat meal when the meals were analysed separately (\(\rho = -0.643, p = 0.119\) and \(\rho = -0.786, p = 0.036\) for high and low-fat meals, respectively).

The initial IGFBP-1 concentration correlated with the initial plasma glucose concentration (\(r = 0.679, p = 0.008\) analysing both meals together and \(r = 0.588, p = 0.165,\) and \(r = 0.810, p = 0.027,\) for high and low-fat meals, respectively), but not with the postprandial glycaemic response.

### 4.3.10 Influence of iv insulin infusion

There were no significant associations between AUC \(_{0-240\ min}\) for glucose or AUC \(_{0-240\ min}\) for paracetamol and the iv insulin infusion dose. In patients receiving iv insulin infusions, there was no significant change in GIP, GLP-1, or ghrelin concentrations before meal ingestion. The iv insulin infusion dose did not correlate with postprandial GIP, GLP-1, or ghrelin responses, but it correlated with the reduction in IGFBP-1 from the initial value (\(\rho = 0.667, p = 0.009\)) analysing both meals together, but that correlation only reached statistical significance for the low-fat meal (\(\rho = 0.630, p = 0.129\) and \(\rho = 0.757, p = 0.049\) for high and low-fat meals, respectively).

### 4.4 EATING DISORDERS (V)

Weight and BMI were larger in adolescent males with T1DM compared with controls (\(p = 0.004\) and \(p = 0.01\), respectively). Patients had higher scores than controls on the “Drive for Thinness” subscale (\(p = 0.002\) and controls had higher scores on the “Bulimia” subscale (\(p = 0.01\)). On the other nine subscales of EDI-C, there was no difference between patients and controls. Three males with T1DM admitted insulin omission to lose weight, but all of them denied it at the interview. One of them had a high score on the “Drive for Thinness” subscale. Two patients and one control subject scored \(\geq 14\) on the “Drive for Thinness” subscale. Consequently, five persons were interviewed by the child and adolescent psychiatrist. None of them was diagnosed as having a current ED according to DSM-IV criteria. One interviewed patient may have had an EDNOS earlier, but had now improved. None of the four interviewed patients had any diabetic complication and their mean HbA1c did not differ significantly from that of the other patients.
5 DISCUSSION
5.1 DIETARY INTAKE IN ADOLESCENTS WITH TYPE 1 DIABETES

Adolescents with T1DM have healthier food habits than non-diabetic age- and sex-matched controls even though the parents of the patients in study I have lower educational level than the parents of the controls. Higher socioeconomic family status is usually associated with better food habits [237]. Despite the healthier food habits, adolescents with T1DM are heavier than their peers, which may be a consequence of the intensive insulin treatment used. The healthier food habits may be a result of the nutritional education and advice given at the diabetes clinics.

However, the nutritional education should focus more on fat quality, fibre intake, and total energy intake and expenditure to reduce cardiovascular risk, improve glycaemia, and prevent weight gain. This statement is based on our finding of a higher intake of saturated fat and a lower intake of fibre than recommended, and the above mentioned increased weight.

The intake of carbohydrates, sucrose, and total fat is within current recommendations, indicating that Swedish adolescents with T1DM adhere to certain parts of the food recommendations better than American youth with T1DM, who consume more total fat than recommended and less carbohydrates than controls [16,17]. The fibre intake found in study I is also higher than that found in American adolescents with T1DM, nevertheless it is less than recommended and much lower than that found in Finnish adolescents with T1DM in the 1980’s [19]. On the other hand, the higher intake of saturated fat than recommended found in study I is in accord with the diet of American youth with T1DM [16,17]. The differences in the diet between adolescents with T1DM living in different countries may be due to differences in the indigenous diet, for example, in Finland the consumption of rye bread is traditionally high leading to a high fibre intake, or in the way that nutritional education is given. The influence of the method used for nutritional education on adherence to food recommendations needs to be evaluated in future studies.

The results of the food recording may have been influenced by the low response rate in that part of the study and, as found by others, irrespective of having T1DM or not [268,269], our patients seem to underreport their intake. The total energy intake of our patients correspond to 80 ± 28% of the estimated total energy expenditure.

The differences found between patients with and patients without coeliac disease in food habits probably reflect the adherence to the gluten-free diet in patients with coeliac disease.

The association found between healthier food habits and better metabolic control is in accord with a recent study [26], as is the association between higher fat and lower carbohydrate consumption and poorer metabolic control [24]. A high intake of fat may include a high intake of saturated fat, which may increase insulin resistance [28] and consequently deteriorate metabolic control. It is possible that even more differences in dietary intake between patients with poor and patients with better metabolic control
would have been found if all eligible patients had participated since patients not participating in the FFQ had higher mean HbA1c than participating patients. However, cross-sectional studies can never show any causal relationships. Prospective, longitudinal, randomized studies are needed, but extremely difficult to perform, to demonstrate a causal relationship between dietary intake and metabolic control in patients with T1DM.

5.2 GASTROINTESTINAL SYMPTOMS IN ADOLESCENTS WITH TYPE 1 DIABETES

GI symptoms are common in both adolescents with and without T1DM. The prevalence of individual symptoms is not increased in adolescents with T1DM compared with controls, which is in accord with a previous finding in adolescents [85], but different from findings in adults with T1DM [75,76]. The lack of difference between patients and controls in our study may be due to the shorter duration of diabetes in our patients compared with adult patients.

The possible impact of diabetes on GI symptoms in adolescents is supported by our finding of more weight loss and reflux episodes in patients with poor metabolic control during the last year, and more reflux episodes and vomiting in patients with longer duration. However, weight loss may be a direct consequence of poor metabolic control due to loss of calories in the urine. As we do not find any impact of current metabolic control, type of prandial insulin, use of insulin pump or MDI, or existence of any diabetic complication on the prevalence of GI symptoms, nor an increased prevalence of symptoms in patients with diabetes, we conclude that GI symptoms may not be associated with diabetes during adolescence.

Adolescents with T1DM have an increased prevalence of abnormal EGG readings [74] and delayed gastric emptying [73], but these disturbances may be asymptomatic. This is supported by the finding of only a weak association between gastric emptying rate and GI symptoms in adults [59,63]. However, severe disorders of motility in the gut in both adolescents and adults are probably symptomatic, but severe motility disorders are probably less common in adolescents than in adults with T1DM, due to the shorter duration of diabetes. This may explain the difference between adults and adolescents with T1DM in prevalence of GI symptoms.

We find more symptoms in girls than in boys, which is consistent with findings in adults with T1DM [75], but contradictory to previous findings in children and adolescents with T1DM [85]. We fail to show any significant association between symptoms in patients and socioeconomic variables, which has been reported previously in nondiabetic school children in an ecological study [270].

Patients with both T1DM and coeliac disease may have a lower fibre intake due to the gluten-free diet than patients with T1DM only, which may explain the higher prevalence of constipation in patients with both diseases. An increased fibre intake, and other efforts as well, aiming at preventing and treating constipation in patients with T1DM and coeliac disease must be advocated in the clinical setting.
Cigarette smoking is associated with gastric and duodenal ulcers, impaired ulcer healing, increased ulcer recurrences, and gastric carcinomas [271,272]. However, associations between smoking and GI symptoms have not been investigated that much. In adults with T1DM, smoking is associated with weight loss and vomiting [76]. We find that adolescents with T1DM smoking daily have more often poor appetite, weight loss, an uncomfortable feeling of fullness at meals, swallowing difficulties, belching, and nausea compared with diabetic adolescents not smoking daily. Meanwhile, nondiabetic adolescents smoking daily only report more vomiting than other controls. This indicates that the co-existence of T1DM and cigarette smoking may aggravate GI symptoms in adolescents. This has never been reported before and needs future investigations for confirmation.

The association between GI symptoms and poor food habits, both irregular meal pattern and avoidance of some healthy foods, found in study II, is in accord with previous findings in nondiabetic children [84]. Whether there is a causal relationship between food habits and GI symptoms in children and adolescents with and without T1DM is not known, but the healthier food habits in adolescents with T1DM found in study I may reduce their prevalence of GI symptoms.

Even though a chronic disease such as T1DM is associated with an increased risk for psychiatric disorders in youth [81] and psychological disorders associate with GI symptoms [83], adolescents with T1DM do not report a higher prevalence of GI symptoms than healthy controls. This indicates that either (1) the mental health of the patients is not inferior to that of their peers or (2) the psychological distress in the patients does not give rise to GI symptoms. The former explanation is supported by findings in study V (see below).

**5.3 POSTPRANDIAL RESPONSES AND GASTRIC EMPTYING**

We describe for the first time the effects of fat supplementation to a meal on postprandial glycaemic response and gastric emptying in patients with T1DM. Fat reduces the initial (0-120 min) glucose excursion and delays gastric emptying. Furthermore, changes in glucose concentrations correlate with simultaneous changes in paracetamol concentrations, the measurement we used for estimating gastric emptying, indicating that the delayed gastric emptying may be, at least partly, responsible for the delayed glucose increase. These findings are in accord with findings in healthy adults and in patients with T2DM [89-91,93,94].

Our findings will influence clinical practice since postprandial normoglycaemia is of great importance for optimal metabolic control [97] and for reducing the risk for diabetic complications, perhaps mainly macrovascular complications [98]. In order to achieve postprandial normoglycaemia, the prandial insulin dose needs to be adjusted not only to the carbohydrate, but also the fat and energy content of the meal. The glucose-lowering effect of the prandial insulin dose needs to come later and probably be prolonged to a fat-rich meal compared with a low-fat meal. Regular human insulin injected just before ingestion of a high-fat meal may be superior to rapid-acting insulin...
analogue. Even a combination of rapid-acting and regular insulin may be useful, as is a
dual-wave bolus dose using CSII (70% of the bolus dose given 10 min prior to the meal
and the rest given continuously during two hours) [273]. Another alternative may be a
repeated dose of a rapid-acting insulin analogue. This needs to be investigated in future
studies.

Our findings of a delayed gastric emptying and a reduced initial glycaemic excursion
after a fat-rich meal will probably also influence food intake in patients with T1DM. In
situations requiring a rapid rise in plasma glucose concentration, the oral intake should
not be high in fat. Prevention of nocturnal hypoglycaemia may also be achieved by
supplementing the bedtime snack with fat. However, nocturnal hypoglycaemia should
primarily be prevented by choosing a suitable insulin regimen and an adequate insulin
dose.

We also describe for the first time in patients with T1DM the effects of fat
supplementation on postprandial responses of GIP, GLP-1, ghrelin, and IGFBP-1. The
total GIP and the early GLP-1 responses are more pronounced after a high-fat than after
a low-fat meal in adolescents with T1DM. This is similar to previous results in adults
with and without T1DM ingesting meals with different energy content but the same
meal composition [109].

Ghrelin decreases after meal ingestion in adolescents with T1DM, and the suppression
is more pronounced after a meal with higher energy and fat content, which is similar to
previous findings in healthy adults [158]. However, our findings are in contrast to those
of Holdstock et al [193]. They did not detect any postprandial ghrelin suppression in
adolescents with T1DM for less than one year. The reason for that may be that they
only determined the ghrelin concentration at one postprandial time point. It is also
possible that differences in duration of diabetes between the study populations may
have affected the results.

Given that patients lacking endogenous insulin secretion were studied, that
euglycaemia was ensured before the ingestion of the meals, and that a standard prandial
insulin dose was given, the differences in GIP, GLP-1, and ghrelin responses found in
study IV cannot be attributed to differences in insulin levels. In contrast, IGFBP-1,
which is predominantly insulin-regulated, decreases before meal ingestion starts in
patients receiving iv insulin infusion and the postprandial decrease does not differ
between meals even though the energy content is twice as high in the high-fat as in the
low-fat meal.

The lower initial glucose concentrations found after the high-fat meal may seem
beneficial for adolescents with T1DM. However, the high fat intake also has negative
effects. One potential negative effect is the pronounced GIP response seen after the
high-fat meal, given that GIP is lipogenic [115] and adolescents with T1DM already
have increased body weight compared with healthy peers [5,6].

The pronounced GIP response seen after the high-fat meal may not be due to the large
fat content per se, but to the large energy content of that meal, since similar responses
to isocaloric meals consisting of either carbohydrates or fat have been found previously
in healthy young adults [108], as well as higher responses after a meal with higher energy content [109].

The difference in GLP-1 response between the test meals is related to the timing of the increase rather than to the total quantity of hormone secreted. The high-fat meal gives rise to a larger early GLP-1 response compared with the low-fat meal. In accordance, we find an association between larger early GLP-1 response and delay in gastric emptying. The limited number of patients studied does not allow us to establish this finding separately for each meal. The association between endogenous GLP-1 secretion and gastric emptying has not been reported before in patients with T1DM, but is in accord with findings in healthy adults [274] and with the effect of exogenous GLP-1 on gastric emptying [47,143]. Our finding is consistent with the concept that GLP-1 secretion influences gastric emptying rather than being influenced by it. The larger early GLP-1 response seen after the high-fat meal is probably important for the delay in gastric emptying seen after that meal and should consequently lead to an attenuated initial glycaemic response, just like that we found in study III. However, a direct association between GLP-1 and glycaemic response is not detected.

Even though we report for the first time that ghrelin decreases significantly after meal ingestion in adolescents with T1DM, the postprandial suppression may be both smaller and delayed compared with the suppression found in healthy adults [157,158]. This needs to be clarified in future, controlled studies. Subnormal postprandial ghrelin suppression in adolescents with T1DM may be due to their increased insulin resistance or their elevated GH levels, since obese adults with insulin resistance and patients with acromegaly lack postprandial ghrelin suppression [168,184]. If subnormal postprandial ghrelin suppression is confirmed in adolescents with T1DM it may have consequences for their increased weight.

The association found between high fasting ghrelin concentrations and low postprandial glucose concentrations supports previous reports that ghrelin is associated with insulin sensitivity [166,175]. High fasting ghrelin levels are significantly associated with high insulin sensitivity. Since all subjects in our study had similar baseline glucose values, ingested the same quantity of carbohydrates, were given the same prandial insulin dose, not adjusted for individual insulin sensitivity, and their postprandial glycaemic responses differed largely and consistently after the meals, a larger postprandial glucose increase probably reflects higher insulin resistance, which would be associated with lower ghrelin levels, just like what we do find in study IV. Future studies should confirm that fasting ghrelin levels are determined by insulin sensitivity in adolescents with T1DM and investigate the involved mechanisms.

A higher fasting IGFBP-1 concentration is associated with a higher fasting plasma glucose concentration in adolescents with T1DM. A similar association is not found for postprandial glucose concentrations, indicating that hepatic insulinization, of which IGFBP-1 is a marker, is more important for fasting than for postprandial glycaemia. Peripheral insulin sensitivity may be of greater importance for postprandial glycaemia.

One of the limitations in these studies is the use of an iv insulin infusion prior to meal ingestion. But gastric emptying, which influences postprandial glycaemia and
potentially hormonal responses to meals, is significantly affected by pre-meal glucose levels [65,69], making it essential to have normoglycaemia at baseline. However, GIP, GLP-1, and ghrelin do not show any acute changes in response to iv insulin infusion nor any obvious delayed effects influencing postprandial responses. Neither are the postprandial glucose and paracetamol concentrations associated with the iv insulin infusion dose. In contrast, we find that the iv insulin dose has impact on IGFBP-1, a protein known to be regulated by insulin.

The test meals used can be compared with findings in study I. The mean energy intake for breakfast was 368 kcal in our patients, 554 kcal for lunch, 578 kcal for dinner, and 666 kcal for snack. The total daily mean energy intake was 2146 kcal. Thus, the energy content of the low-fat meal (320 kcal) was similar to that of an average breakfast eaten by our patients, while the energy content of the high-fat meal (640 kcal) more resembled that of an average dinner or that of every snack eaten during the day. The carbohydrate, protein, and fat content of the meals used in studies III and IV were 75 E%, 19 E%, and 6 E%, respectively, in the low-fat meal, and 38 E%, 9 E%, and 53 E%, respectively, in the high-fat meal. Both these compositions differed from the average composition calculated from all four recorded days (carbohydrate: 53 E%, protein: 16 E%, fat: 31 E%), except from protein content of the low-fat meal. But it cannot be excluded that individual meals with compositions similar to those used in studies III and IV are eaten now and then by adolescents with T1DM.

The fat contents of our test meals (6 E% vs 53 E%, respectively) may not be the most common, but they do exist outside scientific experiments. For example, a meal consisting of pure meat, boiled potatoes, and vegetables may contain only 6 E% fat, while a meal consisting of sausages, fried potatoes, and mayonnaise can contain as much as 69 E% fat and 650 kcal. However, most meals probably have a more moderate fat content and the international recommendation for average, long-time intake is 30–35 E% fat for adolescents with T1DM. Thus, adjustment of the prandial insulin dose to the fat and energy content of the meal may only be needed when the fat content is far from that recommendation. Furthermore, we cannot say whether the differences found between the test meals are due to the addition of fat per se or to the addition of energy, since both fat and energy content differed between our test meals. However, it is not possible to add fat without adding energy, if protein and carbohydrate content are to be the same. The important findings are that the glycaemic and hormonal responses differed between the meals, even though the carbohydrate content, the prandial insulin dose, and the pre-meal glucose concentrations were the same at both meals.

5.4 DIETARY INTAKE, GASTRIC EMPTYING, HORMONAL RESPONSES, SYMPTOMS, AND EATING DISORDERS

In study I, we find that 61% of adolescents with T1DM consume hamburgers, pizza, kebab, and similar fast foods several times per month. This food habit does not differ from that in healthy adolescents. Thus, adolescents with T1DM often consume meals with a high fat content, which will prolong their gastric emptying rate, as found in study III, and, depending on how well they can adjust their prandial insulin dose, affect their postprandial glycaemia. Probably most of them will have early hypoglycaemia
followed by late hyperglycaemia after a fat-rich meal, which is different from healthy adolescents. These abnormalities in glycaemia may cause GI symptoms, since hyperglycaemia is found to augment perceptions from the gut [78,79], and both hypo- and hyperglycaemia acutely affect GI motility. However, the prevalence of GI symptoms does not differ between adolescents with and without T1DM (study II).

As noted before, adolescents with T1DM are heavier than controls (studies I, II, and V). GIP, GLP-1, and ghrelin influence body weight through actions on appetite, lipogenesis, and substrate utilization. Thus, one can speculate that disturbances in postprandial regulation of these hormones may be involved in the pathogenesis of overweight in this patient group. However, our findings in study IV do not support such a speculation since the responses found do not seem to be severely pathological. But that statement is uncertain, as we did not directly compare our findings with those in healthy, matched control subjects.

Some of the GI symptoms asked for in study II may be linked to actions of GLP-1 or ghrelin (poor appetite, loss of weight, symptoms of delayed gastric emptying). Interpreting our GLP-1 and ghrelin findings (study IV) as quite normal would be consistent with the lack of difference found in study II between patients and controls in prevalence of such symptoms.

Whether disturbances in secretion or degradation of GI hormones are of importance for the development of EDs is not known. On the other hand, it is likely that EDs may cause changes in the regulation of GI hormones as EDs affect many other endocrine systems.

5.5 EATING DISORDERS AND ADOLESCENT TYPE 1 DIABETES

We do not find an increased prevalence of EDs in adolescent boys with T1DM compared with age-matched nondiabetic boys. This is opposite to findings in females with T1DM [221] and may be due to gender differences or low statistical power in study V. As EDs are more common in otherwise healthy females than in males [216], one can expect that there may be a gender difference also in patients with T1DM.

The issue of the sample size is important. According to a power calculation performed after the completion of study V, we would have needed 332 patients and as many controls to detect a statistically significant difference in prevalence of EDs, assuming the prevalence to be 1% in the control group and 5% in the patient group. In study V, we only investigated 109 patients and 139 controls. That sample size is large enough for finding a prevalence of 11% in the patient group to be statistically significant different from a prevalence of 1% in the control group. Even though the sample size in study V is small, it is still the largest study published that investigates EDs in adolescent males with T1DM using a two-step procedure.

The eligible but not participating patients in study V had higher mean HbA1c than the participating ones. As EDs are associated with poor metabolic control, it is possible that
we would have found more differences between patients and controls if all eligible patients had participated in the study.

Many females with T1DM do not fulfil the diagnostic DSM-IV criteria for an ED, but anyway they have disordered eating behaviours [6,220,221]. We may have found an increased prevalence of disturbed eating behaviours or abnormal weight control practices in adolescent males with T1DM if we had included also these milder forms in our investigation by the use of additional questions. It is possible that there is a need for development of a diabetes-specific instrument investigating EDs and associated milder forms. Some of the answers given by patients with diabetes using existing instruments may simply reflect their adherence to the dietary regimen, while similar answers in otherwise healthy individuals would indicate a disturbance. In addition, insulin omission to lose weight is a diabetes-specific, abnormal behaviour linked to EDs and that issue is not included in existing instruments.

Our finding of a higher drive for thinness in males with T1DM indicate that they are more concerned about body weight and body shape, which may lead to disturbed eating behaviours. Furthermore, the patients have higher weights than the controls, which may also increase the risk for development of disturbed eating behaviours and EDs. However, there is no difference between patients and controls on the psychopathological subscales, indicating that adolescent males with T1DM cope quite well with their situation and that their mental status is not inferior to that of their healthy peers.

Control subjects in study V report more bulimic symptoms than patients. This has been found before [225,275], indicating that bulimic symptoms may to some extent be normal behaviour among healthy adolescent males, whereas males with T1DM may have to suppress bulimic behaviour.

In conclusion, the prevalence of EDs, and especially the prevalence of disturbed eating behaviours, may be increased in adolescent males with T1DM, but future studies with larger sample sizes and inclusion of broader measurements of disturbed eating behaviours are needed.
6 SUMMARY AND CONCLUSION

Adolescents with T1DM have healthier food habits than adolescents without diabetes. Still, adolescents with T1DM, both boys and girls, are heavier than their healthy peers. Poor metabolic control associates with a high intake of fat and a low intake of carbohydrates. The intake of saturated fat is higher and the intake of fibre is lower than recommended.

The nutritional advice to adolescents with T1DM should focus on energy intake and expenditure to prevent and treat weight gain. It should also focus on fat quality and fibre intake to reduce the risk of macrovascular complications and to promote normoglycaemia.

The prevalence of GI symptoms is high in adolescents with T1DM, but not higher than in age- and sex-matched controls. GI symptoms in patients are associated with female gender, daily cigarette smoking, long duration of diabetes, poor metabolic control during the last year, and an irregular meal pattern, but not with presence of diabetic complication, type of prandial insulin, the use of pump or MDI, or socioeconomic status.

GI symptoms in adolescents with T1DM should be investigated and treated as in other people and should not be assumed to be due to their diabetes. However, adolescents with long duration of diabetes, poor metabolic control, and symptoms from the upper gut may have disordered GI motility and their gastric emptying rate should be investigated during euglycaemia.

A meal with a high fat and energy content reduces the glycaemic response during the first two postprandial hours and delays gastric emptying in adolescents with T1DM compared with a low-fat meal. The glycaemic response correlates significantly with the gastric emptying rate. The prandial insulin dose should be adjusted not only to the carbohydrate, but also to the fat and energy content of the meal in order to reach postprandial normoglycaemia.

A fat- and energy-rich meal stimulates mainly the GIP but also the GLP-1 secretion more than a low-fat meal in adolescents with T1DM. A larger postprandial GLP-1 response is associated with a slower gastric emptying rate. The postprandial ghrelin suppression is larger after a high-fat meal compared with a low-fat meal. The fasting ghrelin level is negatively associated with the postprandial glycaemic response, which may be linked to the association between ghrelin and insulin sensitivity. IGFBP-1 declines after insulin administration irrespective of meal ingestion. The fasting IGFBP-1 level is associated with the fasting glucose level.

Adolescent males with T1DM are heavier and have higher drive for thinness than healthy controls, but do not report more psychopathological problems associated with EDs. Whether adolescent males with T1DM more often have an ED compared with healthy males needs to be investigated in future large-scale studies.
Typ 1-diabetes (T1DM) är den näst vanligaste kroniska sjukdomen i barndomen och efter Finland är insjuknandefrekvensen högst i Sverige. År 2007 insjuknade 685 svenska barn under 18 år i T1DM. Idag har ungefär 7 700 svenska barn under 18 år sjukdomen. T1DM beror på upphörd insulinproduktion, vilket leder till förhöjd koncentration av socker i blodcirkulationen. Orsakerna till att insulin slutar produceras är flera och inte fullt kända. Den förhöjda blodsockerkoncentrationen påverkar kroppen negativt både på kort och lång sikt och motverkas därför genom att kroppens underhudsfett tillförs insulin upprepade gånger varje dag, antingen med insulinpennor eller med insulinpump. Trots behandling är sjukligheten och dödligheten ökad för patienter med T1DM. Kostrådgivning är en av hörnstenarna i diabetesbehandlingen eftersom matintag höjer blodsockret och även kan påverka risken för komplikationer till sjukdomen på lång sikt. Hur ungdomar med T1DM följer givna kostråd är dåligt känt liksom kostens betydelse för blodsockerkontrollen.

Måltidens storlek och sammansättning påverkar blodsockret under timmarna efter måltiden. Denna påverkan sker till stor del genom variationer i hur snabbt magsäcken tömmer sitt innehåll till tunntarmen, där absorptionen av födoämnen sker. Detta gäller friska vuxna och vuxna med typ 2-diabetes, men är ofullständigt studerat hos ungdomar med T1DM. Matintag ger också upphov till förändringar i blodkonzentrationen av olika hormoner som bildas i tarmen eller i angränsande organ. Dessa hormoner har betydelse för bl a blodsockernivån, aptiten och magsäckstömningshastigheten. Vi studerade därför hormonerna GIP, GLP-1 och ghrelin samt bindarproteinet IGFBP-1 före och efter två typer av måltider hos ungdomar med T1DM.

Vuxna patienter som haft T1DM under lång tid har oftare symtom från magen jämfört med friska vuxna. En anledning till detta kan vara störningar i magtarmkanalsrörelserna, antingen till följd av akuta blodsockerförändringar eller kroniska skador i tarmen pga långvarigt förhöjd blodsockernivå. Tidigare har man inte såkert vetat om även ungdomar med T1DM har mer symtom från magen än friska jämnåriga.

Den psykiatriska diagnosen åtstörning samt de mildare formerna av stört åtbetande är vanligare hos tonårsflickor och unga kvinnor med T1DM jämfört med friska kvinnliga jämnåriga. T1DM anses vara en riskfaktor för att utveckla åtstörning pga att sjukdomen och dess behandling kan ge övervikt och pga fokuseringen på vad patienten bör åta. Patienter med T1DM och åtstörning har sämre blodsockerkontroll och ökad risk för diabeteskomplikationer än patienter utan åtstörning. Förekomsten av åtstörning hos tonårspojkar med T1DM är inte känt.

Denna avhandling handlar om dessa olika aspekter av T1DM hos ungdomar och omfattar tre populationsbaserade studier med friska kontroller och två experimentella studier med cross-over design. I de populationsbaserade studierna ingår frågeformulär till samtliga deltagande patienter och kontroller samt kostregistrering respektive intervju av särskilt utvalda individer.
Vi fann att ungdomar med T1DM har bättre matvanor än friska ungdomar. Trots detta väger de mer. Patienterna följer givna kostrekommanderan ganska bra, men intaget av mättat fett är högre och intaget av fiber är lägre än rekommenderat. Patienter som har dålig blodsockerkontroll äter mer fett och mindre kolhydrater än patienter som har bättre blodsockerkontroll. Kostrådgivningen till ungdomar med T1DM bör fokusera mer på energiintag och energiutnyttjande för att förhindra och behandla övervikt. Den bör också fokusera mer på kvaliteten i fettintaget och på fiberintagets storlek, så att risken för hjärtkärlsjukdomar minskar och för att förbättra blodsockerläget.

Ungdomar med T1DM har ofta symtom från magen, men inte oftare än ungdomar utan diabetes. Symtomen hos patienterna har samband med kön (oftare symtom hos flickor), daglig cigarrettrökning, att ha haft diabetes länge, dålig blodsockerkontroll senaste året och oregelbunden måltidsordning. Däremot spelar socioekonomiska faktorer och typ av måltidsinsulin inte någon roll för förekomsten av symtom från magen hos ungdomar med T1DM. Magarmsymtom hos ungdomar med T1DM bör utredas och behandlas förutsättningslöst, precis som hos andra personer, och inte antas bero på diabetesjukdomen. Men ungdomar som haft diabetes i många år, som har dålig blodsockerkontroll och symtom från övre delen av magtarmkanalen bör utredas avseende magsäckstömningshastighet.

En måltid innehållande mycket fett och kalorier ger lägre blodsocker de första två timmarna efter måltiden och långsammare magsäckstömnning hos ungdomar med T1DM jämfört med en fettsnål måltid med lägre kaloritömte. Blodsockernivån efter måltid har starkt samband med magsäckstömningshastigheten. Insulindosen till en måltid bör anpassas, inte bara efter dess kaloritömte, utan även efter dess fett- och kaloritömte, för att normalt blodsocker ska uppstå efter måltiden.

Efter både en fettrik och en fettsnål måltid sker påtagliga förändringar av GIP-, GLP-1- och ghrelinkoncentrationerna i blodet, men förändringarna är mer uttalade efter den fettrika måltiden. IGFBP-1 sjunker efter att insulin getts oberoende av typ av måltid. Ghrelin och IGFBP-1 har samband med blodsockervärden och GLP-1 har samband med magsäckstömningshastighet. Ett stort fett- och kaloritömte i en måltid kan verka gjennomsnittligt hos båda grupper hos ungdomar med T1DM kommer senare och är kanske också mindre än vad som tidigare rapporterats hos friska vuxna, vilket kan bero på deras ökade motstånd mot insulin eller deras ökade nivåer av tillväxthormon. Detta behöver undersökas närmare med friska kontrollpersoner inkluderade i studierna.

Tonårspojkar med T1DM rapporterar högre grad av viktfobi och väger mer än tonårspojkarna utan diabetes. Däremot rapporterar de inte mer psykologiska avvikelse associerade till åtstörningar. Tonårspojkar med T1DM kan ha en ökad risk för att utveckla åtstörning och det bör undersökas närmare i större studier.
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The Medical Library at Örebro University Hospital for their extremely service-minded help and assistance in sending me copies of different articles that I could not access through the Internet.

The Swedish National Food Administration for providing free portion guides.

My parents, Gun-Britt and Jan-Einar Svensson, for love and support, and for taking care of Axel when I ran out of time. And Dad also for painting the picture on the front page of this book.

My son Axel for being such a wonderful boy and for giving me so much love and joy.

My husband Magnus, my very best friend and greatest support in life, for your love, encouragements, patience, and interest in this work.
9 REFERENCES


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Food habits, energy and nutrient intake in adolescents with Type 1 diabetes mellitus

M. Lodefalk and J. Åman*

Abstract

Aims The aims were to describe the food habits of adolescents with Type 1 diabetes (Type 1 DM) and to compare them with healthy control subjects; to describe the distribution of energy-providing nutrients in patients and compare it with current recommendations and previous reports; and finally, to investigate associations between dietary intake and glycaemic control.

Methods One hundred and seventy-four adolescents with Type 1 DM and 160 age- and sex-matched healthy control subjects completed a validated food frequency questionnaire, and 38 randomly chosen patients completed a prospective 4-day food record.

Results Patients ate more regularly, and more often ate fruit and fruit juice, potatoes and root vegetables, meat, fish, egg, offal and sugar-free sweets than control subjects. Control subjects more often ate ordinary sweets and snacks. Patients chose coarse rye bread and dairy products with less fat to a greater extent than control subjects. Patients were heavier than control subjects. The intake of saturated fat was higher in patients compared with recommendations and, for boys with diabetes, the intake of protein was higher than recommended. Patients with poorer glycaemic control ate vegetables, fruit and fish less often than patients with better control.

Conclusions The food habits of adolescents with Type 1 DM were healthier than those of control subjects. The intake of energy-providing nutrients was in line with current recommendations and showed improvements compared with previous reports, with the exception of fibre intake. The association between dietary intake and glycaemic control needs further investigation in prospective studies.


Keywords adolescence, diet, glycaemic control, Type 1 diabetes

Abbreviations BMI, body mass index; E%, energy as percentage of total energy intake; FFQ, food frequency questionnaire; FR, food record; MJ, mega-joules; SDS, standard deviation score; Type 1 DM, Type 1 diabetes mellitus
Food habits, energy and nutrient intake in adolescents with Type 1 diabetes • M. Lodefalk & J. Åman

The importance of adequate food intake in the treatment of Type 1 DM is well established. Dietary fibre, especially if soluble, decreases postprandial glycaemic response in children with Type 1 DM [4,5]. Children with poor glycaemic control consume more fat than those with better control, and an association is observed between saturated fat consumption and insulin dose [6]. In adult male patients, the adjusted fat intake is associated with glycaemic control [7].

Our knowledge of dietary intake among children and adolescents with Type 1 DM is poor. Some authors report fairly good adherence to recommendations [8–11], whereas others report poorer adherence [12–14], i.e. lower intake of carbohydrates and higher intake of fat than recommended. Most of those studies were performed during the 1980s, or earlier, and food recommendations have changed since then. Eating patterns may also have changed. Adherence to recommendations may be influenced by the indigenous diet and seems to decrease with age in adolescents with Type 1 DM [10].

In Sweden, about 20% of healthy adolescent boys and 32% of girls do not eat breakfast every day, and 51% of boys and 61% of girls do not eat the free hot school lunches every school day [15]. The consumption of vegetables is low, but the consumption of sweet foods is high [15,16]. This eating pattern, which shows poor adherence to current recommendations for healthy individuals, may influence adolescents with Type 1 DM.

The aims of this population-based, cross-sectional study were to describe the food habits, i.e. meal pattern and frequency of consumption of different articles of food, in adolescents with Type 1 DM and compare them with those of age- and sex-matched healthy control subjects. We also aimed to describe energy and nutrient intake in adolescents with diabetes and compare it with current recommendations and previous reports. Furthermore, we wanted to investigate relationships between dietary intake and glycaemic control in adolescents with Type 1 DM.

Patients and methods

The study consisted of two parts (Fig. 1). First, all participants completed a food frequency questionnaire (FFQ) and then a subgroup of randomly chosen patients kept a 4-day food record (FR).

Subjects

All adolescents aged 13–19 years, with Type 1 DM for > 1 year, living in the counties of Örebro or Värmland in central Sweden, were identified. For each of the 196 eligible patients, we found an age- and sex-matched healthy control subject at one of two representative schools in Örebro. The counties have a total population of 562,326 [17] and have only one clinic each that provides medical care for children and adolescents with diabetes living in the region.

The demographic, socio-economic and disease-specific variables are shown in Table 1. Patients of both sexes were heavier than their respective control subjects ($P = 0.001$ for girls; $P = 0.002$ for boys). Most parents were gainfully employed, but the educational level was higher in parents of the control subjects than in parents of the diabetic group ($P < 0.001$). More parents of the diabetic group than parents of the control subjects were born abroad ($P = 0.004$ for mothers; $P = 0.001$ for fathers). Sixty-six per cent of the diabetic group and the control subjects were living with both their parents. Ten per cent of the diabetic group had some form of retinopathy, mostly background retinopathy assessed by fundus photography. Only one patient had persistent microalbuminuria, defined as albumin excretion rate > 20 µg/min in an overnight urine collection. Of all eligible patients, 15 (7.7%) had coeliac disease and three
(1.5%) had hypothyroidism. None of the control subjects had coeliac disease or hypothyroidism, but there was no other difference concerning prevalence of co-morbidity. Patients with coeliac disease did not differ from other patients in any respect. The 22 non-participating patients had higher current HbA1c than the participants (8.6 vs. 7.9%; P = 0.028), but did not differ in any other way. There was no difference between randomly chosen patients for the food record and those not chosen, nor was there any difference between patients who completed the food record and those who did not.

The diabetic group was treated in accordance with Swedish guidelines [3] and the nutritional advice given agreed with international guidelines for children and adolescents with Type 1 DM [1].

### Food frequency questionnaire and food record

The diabetic group filled in the FFQ at an ordinary visit to the diabetes clinic and the control subjects completed it at the school nurse’s clinic. The FFQ was based on a questionnaire for healthy adolescents previously used and validated in Sweden [15,16]. We slightly amended it to fit the needs of patients with diabetes. The first questions dealt with the frequency of eating 34 different articles of food such as fat, cheese and meat. In the analyses, these were categorized into 10 food groups: fat; milk and dairy products; vegetables; fruit and fruit juice; potatoes and root vegetables; meat, fish, eggs and offal; bread and cereals; ordinary sweets; sugar-free sweets; and finally, snacks. Snacks comprised potato crisps and cheese doodles, i.e. salty foods with high energy but low nutritional content. Further questions concerned drinks with different meals, percentage of fat in dairy products, type of fat used for home cooking, type of bread, consumption of alcohol, and meal pattern; and, finally, socio-economic and diabetes-specific conditions. The questionnaire contained 73 questions.

The FR subgroup was given detailed information by phone and by mail. They completed the record prospectively on four pre-set days, three weekdays and one weekend day, recording all food and drink they consumed in a food diary, especially constructed for this study. To estimate the amount of ingested foods and drinks, they used ordinary kitchen measures and a portion guide [18]. The portion guide is a tool for improving accuracy and is commonly used in clinical settings and in studies performed by the Swedish National Food Administration. It contains photographs of different portion sizes of different meal components, amount of fat spread on sandwiches and pictures of different sizes of some articles of food, for example, bread, potatoes, fruit and meat. The food records were analysed by a dietician, using a Swedish software program (Dietist 2000; Kost- och Näringsdata AB, Bromma, Sweden).

### Measurement of glycaemic control

HbA1c was measured with high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA). The Mono S standard [19] was used with a reference interval of 3.5–5.3%. Intra- and interassay coefficient of variation was 1.5 and 2.7%, respectively. The results obtained were about 1% unit lower than the Diabetes Control and Complications Trial (DCCT) standard [20]. The same method was used in Örebro and Varmland, and blood samples analysed at both laboratories showed almost

<table>
<thead>
<tr>
<th>FFQ</th>
<th>FFQ</th>
<th>Food record</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetic group</strong></td>
<td><strong>Control subjects</strong></td>
<td><strong>Subgroup</strong></td>
</tr>
<tr>
<td>n = 174</td>
<td>n = 160</td>
<td>n = 38</td>
</tr>
<tr>
<td>Girls (%)</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6 (11.6)</td>
<td>62.1 (10.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.5 (9.1)</td>
<td>170.4 (8.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 (2.9)</td>
<td>21.3 (3.0)</td>
</tr>
<tr>
<td>BMI &gt; 2.5 (s/s) (%)</td>
<td>0.9 (0.9)</td>
<td>0.2 (1.2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.3 (1.7)</td>
<td>16.3 (1.7)</td>
</tr>
<tr>
<td>Parents university graduates (%)</td>
<td>Mothers—31</td>
<td>Mothers—43</td>
</tr>
<tr>
<td>Fathers—22</td>
<td>Fathers—32</td>
<td>Fathers—36</td>
</tr>
<tr>
<td>Born in Sweden (%)</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>Parents born abroad (%)</td>
<td>Mothers—8</td>
<td>Mothers—1</td>
</tr>
<tr>
<td>Fathers—10</td>
<td>Fathers—1</td>
<td>Fathers—21</td>
</tr>
<tr>
<td>Daily smoking (%)</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Current HbA1c (%)</td>
<td>7.9 (1.5)</td>
<td>7.8 (1.5)</td>
</tr>
<tr>
<td>Mean HbA1c last year (%)</td>
<td>7.8 (1.4)</td>
<td>7.6 (1.1)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7.0 (4.0)</td>
<td>7.8 (1.5)</td>
</tr>
<tr>
<td>Daily insulin dose (IU/kg)</td>
<td>1.1 (0.3)</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td>SCII/MDI (%)</td>
<td>16/84</td>
<td>24/76</td>
</tr>
<tr>
<td>Rapid-acting insulin analogue/regular human insulin (%)</td>
<td>76/24</td>
<td>74/26</td>
</tr>
</tbody>
</table>

Values are means with standard deviations in brackets unless noted otherwise. SCII, subcutaneous continuous insulin infusion; MDI, multiple daily injections.
identical mean values (7.9 vs. 8.0%) and very high correlation ($r = 0.932; P < 0.001$).

Statistical analyses and ethics

We used the software program SPSS version 12 (2003; SAS Institute, Cary, NC, USA) for the analyses. Differences in food frequencies were analysed with Mann–Whitney $U$-test. Different proportions were analysed in cross tables and compared using Pearson $\chi^2$ test. Mean values were analysed with independent samples $t$-test and one sample $t$-test, where appropriate. Correlations were analysed with Pearson’s and Spearman’s correlation test, where suitable. We used a multiple linear regression model to analyse confounding factors. The significance level was set at $P < 0.05$.

The Ethics Committee of Örebro County Council approved the study, which was conducted according to the Helsinki ethical rules. Informed consent was obtained from the participants.

Results

Food habits

The diabetic group consumed fruit and fruit juice ($P = 0.006$), potatoes and root vegetables ($P < 0.001$), meat, fish, egg and
Fat  31 (7)  84 (26)  30 (5)  66 (22) < 30 E% 30–35 E%

more often (>1 time/month.

ate hamburgers, pizza, kebab and similar fast foods several per cent of the diabetic group and 66% of the control subjects control subjects drank alcohol at least once weekly. Sixty-one alcohol, but five per cent of the diabetic group and 11% of the diabetic group and 51% of the control subjects never drank alcohol. The diabetic group ate sour milk/yoghurt (>1 time/week; <3 times/week) more often than the diabetic group.

In addition to these differences found in the food groups, we found further differences concerning specific articles of food. The diabetic group ate sour milk/yoghurt (P = 0.001), peas, beans and broccoli (P = 0.019) and porridge (P = 0.031) more often than the control subjects. The diabetic group ate more bread than the control subjects (P = 0.003; 33% eating at least five slices of bread daily compared with 24% of the control subjects. The diabetic group chose coarse rye bread more often than the control subjects and white bread less often. Low-fat butter was used by 81% of the diabetic group and 71% of the control subjects. The diabetic group drank low-fat milk more often than the control subjects (P = 0.001). In the homes of the diabetic group, cooking oil or liquid margarine was most often used for cooking, while in the control subjects' homes, solid butter or solid margarine was used (P = 0.001). About 30% of both the diabetic and control subjects drank at least four glasses of milk daily. Eighteen per cent of the control subjects drank sugary soft drink or juice at least once daily, compared with three per cent of the diabetic group (P = 0.001). Fifty-seven per cent of the diabetic group and 51% of the control subjects never drank alcohol, but five per cent of the diabetic group and 11% of the control subjects drank alcohol at least once weekly. Sixty-one per cent of the diabetic group and 66% of the control subjects ate hamburgers, pizza, kebab and similar fast foods several times/month.

Girls with diabetes ate lettuce, tomatoes and cucumbers more often (P < 0.001), but meat—both as main course (P = 0.005) and on bread (P = 0.020)—less often, than boys with diabetes. Patients with coeliac disease consumed bread (P = 0.012), eggs (P = 0.045) and ordinary buns, cakes and biscuits (P = 0.034) less often and potatoes more often (P = 0.025) than patients without coeliac disease. Patients with coeliac disease ate snacks in the morning more often than patients without coeliac disease (3.5 times/week vs. 2.1 times/week; P = 0.046). The differences between the diabetic and control groups in consumption frequencies did not change if patients with coeliac disease were excluded. The differences in consumption frequencies were not influenced by body mass index (BMI) standard deviation score (SDS), parents’ educational level or origin. The only exception was sour milk/yoghurt.

The diabetic group had breakfast (P = 0.001), morning snack (P < 0.001), dinner (P = 0.001) and evening snack (P = 0.032) more often than the control subjects. About 70% of the diabetic and control groups ate the free hot school lunch every school day.

### Energy and nutrient intake

The FR subgroup ate an average of 5.2 meals daily. Mean daily energy intake was 8.1 (sd = 2.8) mega-joules (MJ; 1938 kcal) and 10.2 (sd = 2.8) MJ (2440 kcal) for girls and boys, respectively. The intake of energy-providing nutrients is shown in Table 3 together with Swedish nutritional recommendations [2] and diabetes-specific recommendations [1]. The intake of protein was higher than recommended in boys (P = 0.040), but not in girls. The intake of saturated fat was higher than recommended in both boys and girls (P = 0.004 and P < 0.001, respectively). The intake of polyunsaturated fat was lower than Swedish recommendations in both boys and girls (P = 0.008 and P = 0.007, respectively). The intake of fibre in girls was lower than the calculated recommendation (P = 0.023). Both male and female patients consumed more protein, less

---

**Table 3** The daily intake of energy-providing nutrients in the FR subgroup and current recommendations

<table>
<thead>
<tr>
<th></th>
<th>Boys n = 16</th>
<th>Girls n = 22</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E%</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>33 (8)</td>
<td>34 (6)</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>42 (30)</td>
<td>35 (6)</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>76 (48)</td>
<td>74 (39)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.8 (5.0)</td>
<td>8.5 (4.0)</td>
</tr>
<tr>
<td>Protein</td>
<td>16 (3)</td>
<td>16 (3)</td>
</tr>
<tr>
<td>Fat</td>
<td>31 (7)</td>
<td>30 (5)</td>
</tr>
<tr>
<td>Saturated</td>
<td>13 (4)</td>
<td>13 (3)</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>11 (3)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>4 (1)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.3 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Fibre</td>
<td>21 (10)</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0 (0)</td>
<td>0.2 (0.5)</td>
</tr>
</tbody>
</table>

**g**

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<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>321 (110)</td>
<td>257 (113)</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>32 (30)</td>
<td>34 (22)</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>48 (48)</td>
<td>42 (24)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>46 (39)</td>
<td>8.5 (4.0)</td>
</tr>
<tr>
<td>Protein</td>
<td>96 (24)</td>
<td>74 (21)</td>
</tr>
<tr>
<td>Fat</td>
<td>84 (26)</td>
<td>66 (22)</td>
</tr>
<tr>
<td>Saturated</td>
<td>36 (12)</td>
<td>29 (9)</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>31 (9)</td>
<td>23 (8)</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>11 (4)</td>
<td>9 (4)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 (0)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Fibre</td>
<td>21 (10)</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0 (0)</td>
<td>0.2 (0.5)</td>
</tr>
</tbody>
</table>

**SNR**

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<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>55–60 E%</td>
<td>&gt; 50 E%</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>30 (26)</td>
<td>30–35 E%</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>46 (22)</td>
<td>&lt; 10 E%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>42 (24)</td>
<td>&lt; 10 E%</td>
</tr>
<tr>
<td>Protein</td>
<td>74 (21)</td>
<td>10–15 E%</td>
</tr>
<tr>
<td>Fat</td>
<td>66 (22)</td>
<td>&lt; 30 E%</td>
</tr>
<tr>
<td>Saturated</td>
<td>29 (9)</td>
<td>≤ 10 E%</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>23 (8)</td>
<td>10–15 E%</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>9 (4)</td>
<td>≤ 10 E%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0 (0)</td>
<td>10–15 E%</td>
</tr>
</tbody>
</table>

**ISPAD**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>21 g</td>
<td>25–35 g†</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>0 (0)</td>
<td>21 g‡</td>
</tr>
</tbody>
</table>

†Recommended for adults.

‡Calculated recommendation for the age group studied.

Values are means with standard deviations in brackets.

ISPAD, International Society for Pediatric and Adolescent Diabetes [1]; SNR, Swedish Nutritional Recommendations [2].
sucrose and more fibre than healthy Swedish adolescents and young adults [16,21] (Table 4).

The energy distribution at different meals did not differ significantly from Swedish recommendations [2] for either girls or boys, although snacks contributed to as much as 30% of total daily energy intake for girls.

Seventy-five per cent of the subgroup followed their normal eating pattern during the days of recording.

### Dietary intake and glycaemic control

Patients participating in FFQ with HbA1c < 7.0% (n = 45; 22 girls) ate peas, beans and broccoli (P = 0.018), fruit and berries (P = 0.029) and fish (P = 0.021) more often, and drank sugar-free juice and soft drinks (P = 0.047) less often than patients with HbA1c ≥ 8.5% (n = 48; 24 girls). Patients with high HbA1c had higher insulin dose than patients with low HbA1c (1.1 vs. 0.9 IU/kg; P = 0.001), were more likely to forget an insulin dose (P < 0.001) and their fathers had a lower educational level (P = 0.018).

Patients in the FR subgroup with HbA1c < 7.0% (n = 11; five girls) consumed less fat (28 vs. 34 E%; P = 0.011) and more carbohydrates (56 vs. 49 E%; P = 0.039) than patients with HbA1c ≥ 8.5% (n = 6; four girls). Parents of subgroup patients with high HbA1c had a lower educational level than those with low HbA1c (P = 0.031 and P = 0.008 for mothers and fathers, respectively).

### Comparison of the food frequency questionnaire and the food record

The concordance between the FFQ and the FR was about 70% for both frequencies of consumption and meal patterns.

### Discussion

There are several methods of estimating dietary intake [22]. Seven-day food records are ‘gold standard’, but are time-consuming and resource-demanding to record and analyse. Twenty-four-hour recall also gives information on energy and nutrient intake, but is the method that yields most under-reporting [23]. A validated FFQ is a useful alternative when large cohorts are investigated [22,24]. FFQs [15,16,25] and semiquantitative FFQs [7,26,27] have been used to study healthy adolescents and patients with diabetes, respectively.

The intake of carbohydrates and total fat found in our FR subgroup agreed with current recommendations, although the intake of saturated fat was higher than recommended. Previously, others have found lower intake of carbohydrates [9–14,28], higher intake of total fat [9,10,12–14,28] and protein [9–11,14] in children and adolescents with Type 1 DM than we found, suggesting an improvement in dietary intake. Higher intake of saturated fat [8], total fat [14,28–30] and lower intake of fibre [30,31] than recommended have been reported previously in children and adolescents with Type 1 DM. This indicates that some dietary education messages may be harder to achieve than others, perhaps because of the indigenous diet. The intake of fibre, unadjusted for energy intake, found in our study was in accordance with the findings of some [8,11,31], but lower than the findings of others [14,32] in patients with Type 1 DM. In all those other studies, patients were younger than our subjects, indicating a poorer fibre intake in our subgroup. However, the fibre intake of our subgroup seems to be higher than that of healthy Swedish adolescents and young adults [16,21]. The intake of sucrose in our study was higher than that found previously in children and adults [16,21].
adolescents with diabetes [10,11,14,28], which may be as a result of a recent more liberal view, allowing sucrose in the diet combined with rapid-acting insulin analogues [1,33]. However, the intake of sucrose in our study did not exceed current recommendations and seems to be lower than that of healthy Swedish adolescents and young adults [16,21].

Differences in food habits between patients and control subjects in our study seem to accord well with differences in nutrient intake between our FR subgroup and previously published data on healthy individuals of the same age. The total energy intake in our subgroup corresponded to 80% (SD = 28%) of the estimated total energy expenditure, which is comparable with other recent results [34,35]. This indicates under-reporting in food records by adolescents with diabetes.

In our study, patients with better glycaemic control reported healthier food habits than those with poorer control. As only a small number kept FRs, the observed associations between nutrient intake and HbA1c are imprecise. However, our finding of a higher fat consumption in patients with poorer glycaemic control is in accordance with other studies [6,7]. Because of the cross-sectional design we cannot say whether glycaemic control depends directly on dietary intake. This needs to be investigated in prospective studies.

Higher socio-economic family status is usually associated with better food habits [15,16]. However, the parents of our diabetic group had lower educational levels than the parents of control subjects, and yet the diabetic group reported healthier food habits. Our diabetic group was also heavier than the control subjects, and yet the diabetic group reported healthier food habits than those with poorer control. As only a small number kept FRs, the observed associations between nutrient intake and HbA1c are imprecise. However, our finding of a higher fat consumption in patients with poorer glycaemic control is in accordance with other studies [6,7]. Because of the cross-sectional design we cannot say whether glycaemic control depends directly on dietary intake. This needs to be investigated in prospective studies.

Higher socio-economic family status is usually associated with better food habits [15,16]. However, the parents of our diabetic group had lower educational levels than the parents of control subjects, and yet the diabetic group reported healthier food habits. Our diabetic group was also heavier than the control group. Higher weight in adolescents with Type 1 DM has been reported before [36–38] and may be as a result of intensive insulin therapy [39–40], although differences in energy intake and physical activity may also contribute [35]. The differences in food habits found in this study between the diabetic group and the control subjects were not influenced by the differences in BMI s.

Ninety-three per cent of our diabetic group was screened at least once for coeliac disease. The prevalence found is in accordance with previous reports [41–44]. Having both coeliac disease and Type 1 DM involves more dietary education and changes. Most of the differences found between patients with and without coeliac disease reflected a natural effort to avoid gluten in patients with coeliac disease.

A limitation of this study is the absence of FRs in the control group. Instead of comparing energy and nutrient intake between the diabetic and control groups, we compared meal pattern and frequencies of food consumption. Energy and nutrient intake in our subgroup was compared with given recommendations and with previously reported findings in patients with Type 1 DM, as well as in healthy adolescents.

We conclude that adolescents with Type 1 DM have healthier food habits than healthy control subjects, which may be a result of nutritional education to the patients. Body weight is not only dependent on eating habits, reflected by our finding of heavier patients than control subjects. The distribution of energy-providing nutrients in our subgroup was consistent with current food recommendations and showed improvements compared with previous reports in children and adolescents with diabetes, except for fibre intake. However, the intake of saturated fat should still be lower. Patients with better glycaemic control had healthier food habits than patients with poorer control. Future studies are needed for investigation of the influence of dietary intake on glycaemic control, insulin sensitivity and risk for acute and long-term complications, as well as studies comparing energy and nutrient intake in individuals with and without diabetes. Nutritional education of adolescents with Type 1 DM needs to focus more on energy intake and expenditure to prevent weight gain and on fat quality to reduce cardiovascular risk.

Acknowledgements

We thank Gudrun Jonsell for inclusion of patients in Värmland, Gösta Samuelson for interesting discussions while planning the study and Scott Montgomery for statistical advice concerning randomization of the FR subgroup. We are grateful to Liss Bryngelsson who helped us with data transformation, and Inga-Lill Detlofsson, Linda Haglund and Susanne Alm for their help with the questionnaire, the food diary and the analyses of the FRs.

Competing interests

We are grateful to the Swedish National Food Administration for providing free portion guides and to the Research Foundation of Örebro and Värmeland for financial support.

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Gastrointestinal symptoms in adolescents with type 1 diabetes

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Abstract

Objective: To investigate associations with and prevalence of gastrointestinal (GI) symptoms in adolescents with type 1 diabetes (T1DM) in comparison with age- and sex-matched controls.

Method: In a population-based, cross-sectional setting, 174 adolescents with T1DM and 160 controls filled out a questionnaire concerning GI symptoms, food habits, socioeconomic, and diabetes-specific variables.

Results: Seventy-seven percent of both patients and controls reported at least one GI symptom. Girls reported significantly more symptoms than boys. Patients with a longer duration than seven years reported more often reflux episodes ($p = 0.046$) and vomiting ($p = 0.006$). Patients with poor metabolic control during the past year reported more often loss of weight ($p = 0.050$) and reflux episodes ($p = 0.011$). Patients smoking daily had more often a poor appetite ($p = 0.024$), loss of weight ($p = 0.039$), an uncomfortable feeling of fullness ($p = 0.004$), swallowing difficulties ($p = 0.005$), belching ($p = 0.026$), and nausea ($p = 0.016$). Patients with an irregular meal pattern experienced more often early satiety ($p = 0.025$). Patients reporting at least one GI symptom consumed less often milk ($p = 0.011$), potatoes ($p = 0.002$), and meat on sandwiches ($p = 0.043$) than other patients.

Conclusions: GI symptoms in adolescents with T1DM are common, but the prevalence is not increased compared with nondiabetic controls, and is associated with gender, duration of diabetes, metabolic control during the past year, food habits, and cigarette smoking, but not with type of prandial insulin, presence of diabetes complication, or socioeconomic status.

Key words: adolescent, diabetes mellitus, type 1, food habits, gastrointestinal tract
**Abbreviations**

**BMI**  Body mass index  
**BMI SDS**  Body mass index standard deviation score  
**CSII**  Continuous subcutaneous insulin infusion  
**CV**  Coefficient of variation  
**DCCT**  Diabetes control and complications trial  
**GI**  Gastrointestinal  
**HbA$_{1c}$**  Glycated hemoglobin  
**MDI**  Multiple daily injections  
**SD**  Standard deviation  
**SPSS**  Statistical Package for the Social Sciences  
**T1DM**  Type 1 diabetes mellitus  
**T2DM**  Type 2 diabetes mellitus
Introduction

Gastrointestinal (GI) symptoms are common in the general population, but it is not clear whether the prevalence is increased in adolescents with type 1 diabetes (T1DM). In adults with long-standing T1DM, GI symptoms are more prevalent than in healthy controls (1,2) and the etiology is probably multifactorial (3). However, some authors do not report an increased prevalence of GI symptoms in adults with T1DM (4-6), which may be due to methodological weaknesses in some of these studies or a short duration of diabetes.

There is only one controlled study investigating the prevalence of GI symptoms in children and adolescents with T1DM, and it did not find an increased prevalence of symptoms (7). However, the patients were not recruited in a population-based setting and the control group did not come from the general population, for which reason the results may be biased. Furthermore, an increased prevalence of abnormal electrogastrography readings has been reported in children and adolescents with T1DM (8), as well as a high prevalence of delayed gastric emptying (9). Since abnormal GI electrical activity and motility are considered to be possible causes of GI symptoms (3), these findings indicate that GI symptoms may also be more prevalent in adolescents with T1DM.

Other possible reasons for an increased prevalence of GI symptoms in patients with T1DM are the effects of acute hyperglycemia and the increased prevalence of celiac disease and psychiatric disorders (10,11). Psychological variables are related to GI symptoms of functional origin in both children and adults (12,13) and celiac disease can elicit such GI symptoms as abdominal pain, diarrhea, constipation, and bloating (14).
Hyperglycemia increases the perception of symptoms from the gut (3), indicating that patients with diabetes may be more sensitive to such symptoms. Acute hyperglycemia, even in the form of physiological postprandial increases in plasma glucose concentrations, also delays gastric emptying (15,16). This delayed gastric emptying may produce the same symptoms from the upper GI tract as found in patients with gastroparesis, which is a chronic form of severely delayed gastric emptying seen more often in patients with long-standing diabetes than in healthy individuals (3). Symptoms of gastroparesis are nausea, vomiting, early satiety, bloating, and abdominal pain (17), and especially these symptoms and others from the upper GI tract, such as an uncomfortable feeling of fullness at or after meals, swallowing difficulties, and reflux episodes, are reported to be more prevalent in adults with long-standing T1DM than in healthy matched controls (1).

On the other hand, the dietary advice given to patients with T1DM may reduce the risk for GI symptoms. Such advice is aimed at promoting normoglycemia in conjunction with insulin therapy, reducing the risks of hypo- and hyperglycemia, and minimizing the risks of micro- and macrovascular complications (18). We have previously shown that adolescents with T1DM have healthier food habits than nondiabetic controls (19) and associations between poor food habits and GI symptoms have been found in nondiabetic children (12). Associations between food habits and GI symptoms have never been investigated in patients with T1DM.

Our hypotheses were that GI symptoms are more prevalent in adolescents with T1DM than in nondiabetic controls even though they have better food habits, and that GI symptoms in patients are associated with poor metabolic control and long duration of diabetes. The aim of this study was to investigate the prevalence of GI symptoms in adolescents with T1DM in a population-based setting and compare it with age- and sex-matched nondiabetic controls from
the general population. An additional aim was to investigate associations between GI symptoms and demographic and socioeconomic variables, food habits, and diabetes-specific variables, such as metabolic control, duration of diabetes, type of prandial insulin, and microvascular complications.
Methods

Study procedure

In this population-based, cross-sectional study, we collected information about GI symptoms in adolescents with T1DM and in nondiabetic matched controls at the same time as we collected information about food habits, and socioeconomic and diabetes-specific variables. The patients filled out a questionnaire at an ordinary visit to the diabetes clinic and the controls completed the same questionnaire at their school nurse reception. The diabetes nurse filled out a form pertaining to current HbA1c and values during the past year, presence of any retinopathy as assessed by fundus photography, or persistent microalbuminuria, defined as an albumin excretion rate of more than 20 µg/min in an overnight urine collection and experience of severe hypoglycemia or diabetes ketoacidosis during the past year.

Subjects

Patients and controls, as well as their food habits, have been described previously (19). The patient group consisted of all patients aged 13-19 years living in the counties of Örebro and Värmland, in central Sweden, with T1DM for more than one year. The control group consisted of nondiabetic pupils at two representative schools in Örebro, and each one of them was chosen from school registers according to sex and age to match one of the patients. One hundred and seventy-four of 196 eligible patients (response rate 89%) and 160 of the eligible controls agreed to participate (response rate 82%). One control was excluded because she reported having a bowel disease. Demographic and diabetes-specific characteristics of the participating patients and controls are shown in Table 1.

Questionnaire
The questionnaire consisted of altogether 87 questions, the last six ones being diabetes-specific and addressed only to the patients. The first 58 questions dealt with food habits, the following 13 dealt with GI symptoms during the last three months and, finally, 10 questions dealt with socioeconomic issues and any disease prevalence. The questions dealing with GI symptoms originated from a postal questionnaire previously validated in a Swedish population (20) and used in respondents with and without T1DM (1). The last question about GI symptoms was open, thus making it possible for patients and controls to describe in their own words any GI symptom not covered by the other questions.

**Analysis**

HbA1c was measured by high-performance liquid chromatography (BIO-RAD Diagnostic Group, CA, USA). The Mono S standard (21) was used with a reference interval of 3.5-5.3%. Intra- and interassay CV were 1.5 and 2.7%, respectively. The results obtained were about 1 percentage unit lower than the DCCT standard (22).

**Statistics and ethics**

The statistical analyses were done in SPSS version 12.0.1 (2003; SAS Institute, Cary, NC, USA). Results are given as the mean (SD) or proportions. Proportions were compared using Fisher’s exact test with two-sided significance. Variables with a normal distribution were compared using the unpaired t test. Food habits in different groups of patients where compared using the Mann-Whitney U test. Statistical significance was set at p < 0.05.

The study sample size was large enough to detect a statistically significant difference between patients and controls using $\alpha = 0.05$, power = 0.80, and some of the proportions derived from Schvarcz et al (1).
The study was approved by the Ethics Committee of Örebro County Council and was conducted according to the Declaration of Helsinki. All subjects gave their informed consent.
Results

Comparison between patients and controls

Both patients and controls reported that they had been troubled during the last three months by, on average, two GI symptoms each. Seventy-seven percent of both patients and controls reported at least one symptom. The proportions of patients and controls reporting individual symptoms did not differ (Table 2). Abdominal pain was the most prevalent complaint in both patients and controls. The proportions of patients and controls reporting at least one symptom from the upper GI tract did not differ either.

Symptoms and demographic and socio-economic variables

More girls than boys reported GI symptoms in both the patient and control groups [patients: abdominal pain (p = 0.013), an uncomfortable feeling of fullness at or after meals (p = 0.003), nausea (p < 0.001); controls: abdominal pain (p = 0.024), early satiety (p = 0.010), nausea (p = 0.005)]. Patients more than 16 years old (n = 98) reported a poor appetite more often than younger patients (p = 0.020). Controls more than 16 years old (n = 86) reported swallowing difficulties more often than younger controls (p = 0.022). Patients with BMI > 23 (n = 86) reported more often than patients with BMI < 23 abdominal pain (p = 0.008) and nausea (p = 0.011). There was no difference in the prevalence of individual symptoms between controls with BMI more or less than 23.

Socioeconomic variables in the patient group, such as ethnic origin of parents, educational level of parents, occupation of parents (working outside home, studying, or on parental leave vs. unemployment, being on the sick list, or retired), and living with both parents or not, were associated only to a minor degree with the prevalence of individual GI symptoms.
Patients smoking cigarettes daily (n = 10) had more often a poor appetite, loss of weight, an uncomfortable feeling of fullness at or after meals, swallowing difficulties, belching, and nausea than patients not smoking daily (p = 0.024, p = 0.039, p = 0.004, p = 0.005, p = 0.026, and p = 0.016, respectively). Controls smoking daily (n = 13) reported more often vomiting (p = 0.007) than controls not smoking daily.

**Symptoms and food habits**

Early satiety was more prevalent and belching and swallowing difficulties tended to be more prevalent in patients not eating breakfast, lunch, and dinner every day (n = 75) compared with patients eating these meals every day (p = 0.025, p = 0.057, and p = 0.071, respectively). Controls not eating breakfast, lunch, and dinner every day (n = 100) more often reported abdominal pain, nausea, and diarrhea than other controls (p = 0.013, p = 0.049, and p = 0.023, respectively).

Patients reporting at least one GI symptom drank milk, ate potatoes, and ate meat on sandwiches less often than patients without symptoms (p = 0.011, p = 0.002, and p = 0.043, respectively). Controls reporting at least one GI symptom ate fish less often than controls without symptoms (p = 0.003).

**Symptoms and diabetes-specific variables**

Patients with a duration of diabetes of more than seven years (n = 91) reported more often reflux episodes (p = 0.046) and vomiting (p = 0.006) than patients with a shorter duration. The prevalence of individual GI symptoms did not differ between patients with a current \( \text{HbA}_{1c} < 7.0\% \) (n = 45) and patients with a current \( \text{HbA}_{1c} \geq 8.5\% \) (n = 47). Loss of weight and reflux episodes were more prevalent in patients with a mean \( \text{HbA}_{1c} \) during the past year \( \geq \)
8.5% (n = 44) than in patients with a mean HbA1c during the past year < 7.0% (n = 44; p = 0.050 and p = 0.011, respectively).

The prevalence of individual symptoms did not differ between patients with a microvascular complication (n = 17) and patients without this complication, nor between patients who had experienced ketoacidosis during the past year (n = 8) and those who had not, or between patients who had experienced a severe hypoglycemic episode during the past year (n = 22) and patients who had not. The type of prandial insulin (rapid-acting insulin analogue or human insulin) did not differ in patients with and without individual GI symptoms. The prevalence of individual symptoms did not differ according to use of an insulin pump or not.

Twelve patients had celiac disease. They reported more often constipation (p = 0.030) than patients without celiac disease. Otherwise, there was no difference in the prevalence of individual GI symptoms between patients with and without celiac disease.
Discussion

In this study we find that GI symptoms are common in adolescents both with and without T1DM. We find that the prevalence of individual symptoms does not differ between patients and controls, which is in accord with a previous finding in adolescents (7), but differs from findings in adults (1,2). The lack of difference in the prevalence of symptoms may not be due to low statistical power in this study, but it may be influenced by the shorter duration of diabetes in our patients compared with adult patients. This is supported by our finding of more reflux episodes and vomiting in adolescents with longer duration. The possible impact of diabetes on GI symptoms is also supported by our finding of more weight loss and reflux episodes in adolescents with poor metabolic control during the past year. On the other hand, weight loss may be a direct consequence of poor metabolic control due to a loss of calories in the urine. Furthermore, we do not find any impact of the type of prandial insulin, use of an insulin pump, or the existence of any diabetic complication on the prevalence of individual GI symptoms.

The questions about GI symptoms used in this study focused on the upper GI tract since it was shown in adults with T1DM that these symptoms were more prevalent than in controls (1). One possible explanation of the increased prevalence of these symptoms may be disordered GI motility, especially delayed gastric emptying associated with long-standing diabetes (3). However, symptoms that may indicate disordered motility in the upper GI tract are not more prevalent in patients than in controls in this study. This finding suggests that disordered motility in the upper GI tract is asymptomatic in adolescents with T1DM or that adolescents with T1DM do not exhibit disordered motility. The latter is contradictory to findings of a high prevalence of abnormal electrogastrography readings (8) and delayed gastric emptying (9) in children and adolescents with T1DM. However, adolescents with T1DM and symptoms of
chronic dyspepsia or chronic constipation were shown to have gastric emptying rates and mouth-to-anus transit times similar to those of nondiabetic adolescents with similar symptoms (23), indicating that GI symptoms in adolescents with T1DM are not related to disordered GI motility. Furthermore, the relationship between gastric emptying rate and GI symptoms is weak in adults (3). Thus, adolescents with T1DM may have disordered GI motility, but it is probably asymptomatic. These assumptions need further investigation.

Our finding of more GI symptoms in girls than in boys is consistent with findings in adults with T1DM (1), but contradictory to findings in children and adolescents with T1DM (7). We failed to show any significant association between GI symptoms in patients and socioeconomic variables, which has been reported previously in nondiabetic schoolchildren (24), indicating that GI symptoms in adolescents with T1DM may not be due to low socioeconomic status.

Patients with both T1DM and celiac disease may have a lower fiber intake than patients with T1DM only, which may explain the higher prevalence of constipation in patients with both diseases. An increase in fiber intake and other efforts as well aimed at preventing and treating constipation in patients with T1DM and celiac disease must be advocated in the clinical setting.

It is a well-known fact that cigarette smoking is associated with gastric and duodenal ulcers, impaired ulcer healing, and increased ulcer recurrences (25,26), but associations between smoking and GI symptoms have not been as thoroughly investigated. We find that daily smoking is associated with an increased prevalence of GI symptoms mainly in the patients,
indicating that the co-existence of T1DM and cigarette smoking may aggravate GI symptoms in adolescents.

Patients in this study reporting at least one GI symptom drank milk less often than other patients, which may be due to lactose intolerance or cow milk allergy. However, no patient reported having such a condition and the association is not found in the control group. The association between GI symptoms and poor food habits, both an irregular meal pattern and avoidance of healthy foods, found in this study is in accord with previous findings in nondiabetic children (12). Whether there is a causal relationship between food habits and GI symptoms in children and adolescents with and without T1DM needs further evaluation.

We conclude that the prevalence of GI symptoms in adolescents with T1DM is high, but not higher than in age- and sex-matched controls from the general population even though T1DM is associated with disordered GI motility, increased prevalence of celiac disease, and psychological disturbances. The adolescent patients’ healthier food habits may reduce the prevalence of GI symptoms. Girls report more symptoms than boys and patients with known celiac disease have more constipation than other patients. GI symptoms in adolescents with T1DM are associated with daily cigarette smoking, poor food habits, poor metabolic control during the past year, and a longer duration of diabetes, but not with current metabolic control, presence of any diabetes complication, type of prandial insulin, use of an insulin pump, or socioeconomic status.
Acknowledgements

We thank Gudrun Jonsell for the recruitment of patients in Värmland, Liss Bryngelsson who helped us with data transformation, and Lars Agrèus for help with the questionnaire. We are also grateful to the Research Foundation of Örebro and Värmland, the Society for Child Care, the Frimurare Barnhuset Foundation in Stockholm, the Research Committee of the Örebro County Council, and the Pediatric Diabetes Foundation for financial support.
Reference List


Table 1. Characteristics of participating patients and controls. Values are means (SD) unless noted otherwise.

<table>
<thead>
<tr>
<th></th>
<th>Patients n = 174</th>
<th>Controls n = 159</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>52.9%</td>
<td>53.5%</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>16.3 (1.7)</td>
<td>16.3 (1.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6 (11.6)</td>
<td>62.2 (10.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.5 (9.1)</td>
<td>170.4 (8.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 (2.9)</td>
<td>21.4 (2.9)</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.9 (0.9)</td>
<td>0.2 (1.1)</td>
</tr>
<tr>
<td>BMI &gt; 2 SD</td>
<td>9.8%</td>
<td>3.8%</td>
</tr>
<tr>
<td>Daily smoking</td>
<td>5.7%</td>
<td>8.2%</td>
</tr>
<tr>
<td>Current HbA₁c (%)</td>
<td>7.9 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Mean HbA₁c past year (%)</td>
<td>7.8 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>7.0 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Daily insulin dose (IU/kg)</td>
<td>1.1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>CSII vs. MDI</td>
<td>16% vs. 84%</td>
<td></td>
</tr>
<tr>
<td>Rapid-acting insulin analogue vs. regular human insulin</td>
<td>78% vs. 22%</td>
<td></td>
</tr>
<tr>
<td>Any form of retinopathy</td>
<td>10.1%</td>
<td></td>
</tr>
<tr>
<td>Persistent microalbuminuria</td>
<td>0.8%</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Proportions of patients and controls (%) answering yes to the question, “Have you been troubled during the last three months by…?”

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>39.3</td>
<td>45.9</td>
<td>NS</td>
</tr>
<tr>
<td>Poor appetite</td>
<td>24.9</td>
<td>27.0</td>
<td>NS</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>4.9</td>
<td>7.1</td>
<td>NS</td>
</tr>
<tr>
<td>Uncomfortable feeling of fullness at or after meals</td>
<td>21.5</td>
<td>15.6</td>
<td>NS</td>
</tr>
<tr>
<td>Swallowing difficulties</td>
<td>4.7</td>
<td>6.3</td>
<td>NS</td>
</tr>
<tr>
<td>Belching</td>
<td>27.3</td>
<td>23.9</td>
<td>NS</td>
</tr>
<tr>
<td>Reflux episodes</td>
<td>14.0</td>
<td>15.9</td>
<td>NS</td>
</tr>
<tr>
<td>Early satiety</td>
<td>35.5</td>
<td>43.3</td>
<td>NS</td>
</tr>
<tr>
<td>Nausea</td>
<td>28.3</td>
<td>30.2</td>
<td>NS</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5.8</td>
<td>7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Constipation</td>
<td>11.0</td>
<td>8.2</td>
<td>NS</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>15.7</td>
<td>15.8</td>
<td>NS</td>
</tr>
<tr>
<td>Other abdominal symptoms</td>
<td>6.3</td>
<td>6.9</td>
<td>NS</td>
</tr>
</tbody>
</table>
Original Article: Treatment

Effects of fat supplementation on glycaemic response and gastric emptying in adolescents with Type 1 diabetes

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Abstract

Aims To compare the glycaemic response to meals with different fat content in adolescents with Type 1 diabetes mellitus (T1DM) and to investigate associations with gastric emptying.

Methods In this randomized, cross-over study, paired results were obtained from seven adolescents with T1DM who ingested on different days two meals with the same carbohydrate and protein content, but different fat and energy content (2 and 38 g fat, 320 and 640 kcal, respectively). Paracetamol was mixed into the meals and gastric emptying was estimated by the paracetamol absorption method. All subjects were normoglycaemic and given 7 IU insulin aspart at commencement of ingestion. Postprandial blood samples were taken during 4 h.

Results The areas under the curves for plasma glucose and serum paracetamol concentrations were larger after the low-fat than after the high-fat meal during the first 2 h (P = 0.047 and P = 0.041, respectively). The difference between meals in time-to-peak in glucose and paracetamol concentrations did not reach statistical significance (high-fat vs. low-fat meal: 210 min (120–240) vs. 120 min (50–240), P = 0.080 and 120 min (75–180) vs. 60 min (60–120), P = 0.051, respectively). Changes in glucose concentrations correlated with simultaneous changes in paracetamol concentrations (P < 0.001).

Conclusions For the first time, we have shown that the initial glycaemic response is reduced after a meal with higher compared with a meal with lower fat content in adolescents with T1DM given a rapid-acting insulin analogue preprandially. The type and dose of preprandial insulin may need adjustment to the fat content of the meal to reach postprandial normoglycaemia.


Keywords adolescents, dietary fat, gastric emptying, postprandial glycaemic response, Type 1 diabetes

Abbreviations AUC, area under the curve; BMI, body mass index; CSII, continuous subcutaneous insulin infusion; CV, coefficient of variation; E%, energy per cent; HbA1c, glycated haemoglobin; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus

Introduction

It is well known that poor glycaemic control is associated with an increased risk of long-term complications in patients with Type 1 diabetes mellitus (T1DM). Both fasting and postprandial glucose levels influence control, and in the lower range of glycated haemoglobin (HbA1c) postprandial glucose levels contribute more to its elevation than fasting glucose levels, at least in patients with Type 2 diabetes mellitus (T2DM) [1]. Postprandial glucose levels are influenced by several factors. Insulin lispro lowers postprandial glucose excursions more than regular insulin in children and adolescents with T1DM [2,3] and after an energy-rich meal, high in carbohydrates and fat, a dual wave of rapid-acting insulin given by continuous subcutaneous insulin infusion (CSII) leads to lower glucose levels than other types of bolus doses in patients with T1DM [4].

Preprandial glucose levels, insulin sensitivity and the carbohydrate content of the meal also affect postprandial glucose levels in patients with T1DM [5]. In patients with T2DM and in healthy individuals, the fat content of the meal also influences postprandial glycaemia [6,7], but the effect of dietary...
fat on glycaemic response has not been investigated in patients with T1DM.

The influence of the meal content on glycaemic response seems to be mediated, at least partly, by changes in gastric emptying. The importance of the gastric emptying rate for postprandial glycaemia was highlighted by Horowitz et al., recently [8]. The gastric emptying rate in healthy adults is dependent on the nutritive density of the meal [9], and gastric emptying of carbohydrates correlates to postprandial glycaemic response in healthy adults [10] and in adults with T1DM and T2DM without pronounced postprandial hyperglycaemia [11]. Patients with T1DM and gastroparesis require less insulin in the first two postprandial hours than those with T1DM and normal gastric emptying [12]. On the other hand, gastric emptying rate is influenced by the plasma glucose concentration. It is increased by hyperglycaemia [13–15] and decreased by hyperglycaemia [16,17] in both healthy individuals and in patients with T1DM.

Studies on postprandial glycaemia and gastric emptying in paediatric populations with T1DM are lacking. Previous studies on adult patients have often included both T1DM and T2DM populations; have included T1DM patients with long duration and thus potential autonomic dysfunction; and have not controlled for fasting glucose levels or standardized the prandial insulin dose. All these factors may affect gastric emptying, as well as the postprandial glucose responses.

In this study, we compared the postprandial glycaemic responses to meals with the same carbohydrate and protein content, but different fat and energy content, in adolescents with T1DM given a fixed preprandial insulin dose. We also investigated associations between glycaemic responses and a simple measure of gastric emptying.

**Patients and methods**

**Patients**

Ten adolescents (five girls) with T1DM for at least 2 years with modest glycaemic control and no signs of remaining remission were recruited from the out-patient diabetes clinic at Astrid Lindgren Children’s Hospital, Karolinska University Hospital (Stockholm, Sweden). Exclusion criteria were coeliac or other gastrointestinal disease, major gastrointestinal symptoms, prior abdominal surgery except appendectomy, any diabetes complication such as retinopathy, microalbuminuria or hypertension, and any medication other than insulin. One patient withdrew consent and two patients were excluded from the study (one because he developed postprandial hypoglycaemia and one because he participated only once). The remaining seven subjects’ mean age was 16.4 ± 0.7 years (range 15.5–17.5), body mass index (BMI) 23.2 ± 3.4 kg/m² (range 18.8–29.3), diabetes duration 3.7 ± 1.2 years, HbA₁c 7.3 ± 0.7% (Monro-S standard) and insulin dose 0.8 ± 0.2 IU kg⁻¹ day⁻¹. All subjects were in Tanner stage 5. They were all treated with multiple insulin injections using basal insulin glargine once daily before evening meal or at bedtime and insulin aspart or insulin lispro before every meal and snack.

**Test meals and assessment of gastric emptying**

The test meals were prepared in advance by two dieticians and stored at −20°C. The meals consisted of pasta with a sauce of tomatoes and ham with or without rape seed oil. The total energy content was 320 and 640 kcal, and the fat content 20 kcal [2 g, 6 energy per cent (E%)] and 340 kcal (38 g, 53 E%) in the low- and high-fat meal, respectively. Both types of meal contained the same amount of carbohydrates (240 kcal, 60 g) and protein (60 kcal, 15 g). The subjects were allowed to drink 100 ml of water together with the meal. The meal was consumed in 15 min in a sitting position. Gastric emptying was assessed by the paracetamol absorption method [18–23]. Paracetamol (30 mg/kg, Alvedon® tablet, AstraZeneca Sverige AB, Södertälje, Sweden) was pulverized in a mortar and carefully mixed into the test meals.

**Study procedure**

The seven participating subjects ingested both meals in random order on different days separated by 6–14 days. They came to the diabetes clinic at 08.00 h after fasting overnight and had not taken any insulin since the previous evening. An intravenous (i.v.) cannula was placed in the antecubital vein of each arm. One arm was warmed by heating pads for arterialization of the blood and used for analyses of plasma glucose [24].

The subjects needed to be normoglycaemic at baseline, i.e. when ingestion of the meal began, since both hyper- and hypoglycaemia affect gastric emptying rate. Therefore, a variable i.v. insulin infusion (0.02–0.2 IU kg⁻¹ h⁻¹) was commenced prior to baseline in subjects with hyperglycaemia (> 7.5 mmol/l). If a subject had hypoglycaemia (< 4.0 mmol/l) the test was not performed that day. Before the low-fat meal, six of the subjects received an insulin infusion, with 3.6 IU (2.5–17.3) given over 42.5 min (30–110). Before the high-fat meal, four of the subjects received an insulin infusion, with 4.0 IU (0.3–5.8) given over 37.5 min (5–85). A rest period of at least 30 min separated the end of the insulin infusion and the commencement of meal ingestion.

At baseline, a subcutaneous injection of insulin aspart 7 IU [25] was given laterally to the umbilicus in the same place on both occasions. Blood samples were taken at baseline and 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210 and 240 min after baseline. All subjects remained in a sitting position during the whole study period.

**Analyses**

HbA₁c was measured by an immunochemical method (Cobas Integra 400; Roche Diagnostics Scandinavia AB, Bromma, Sweden), and the Mono-S standard [26] was used. HbA₁c standardized according to the National Glycohaemoglobin Standardization Program equals 0.92 times HbA₁c measured with the Mono-S standard plus 1.33. The reference interval was < 5.2% and the intra- and interassay coefficient of variation (CV) < 3%. Plasma glucose concentrations were measured using bedside equipment based on a glucose dehydrogenase method (HemoCue B-Glucose Analyzer; HemoCue AB, Angelholm, Sweden) during the insulin infusion to manage the infusion rate. Baseline and postprandial plasma glucose concentrations
were measured by a glucose oxidase-based method (Synchron LX20; Beckman Coulter AB, Bromma, Sweden) with intra- and interassay CV < 4%. The serum paracetamol concentrations were measured using fluorescence polarization immunoassay technology (TDx/TDxFLx; Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA). The detection limit was 1.00 µg/ml (6.6 µmol/l) and the intra- and interassay CV was < 5%.

Statistics and ethics

Results are given as mean ± SD if normally distributed, otherwise as median (range), unless otherwise stated. The area under the curve (AUC) was calculated according to the trapezium rule. Comparisons between the test meals were made with paired samples t-test and the Wilcoxon signed ranks test, where appropriate. Correlations were done with Pearson and Spearman’s ρ, where appropriate. To investigate relationships between glucose and paracetamol concentrations, we used ANOVA for repeated measurements with differences in glucose concentrations between measurements at 90 min and baseline and at 240 min and 90 min as the outcome. Three explanatory variables were used in the model: (i) differences in paracetamol concentrations between the same time points, (ii) time points (90–0 min and 240–90 min), and (iii) type of meal. The repeated measurements ANOVA was computed as the mixed model and implemented in the procedure MIXED in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The other statistical analyses were performed in SPSS version 12.0 (SPSS Sweden AB, Sundbyberg, Sweden). Statistical significance was set at P < 0.05.

In the power calculation performed prior to the study, the primary endpoint was a difference between test meals in plasma glucose concentration of at least 2.5 mmol/l at any time point. With the study design used, with a probability of finding that difference of 90%, significance level 0.05, and SD 1.6, 10 subjects were required.

The study was approved by the Regional Council of Ethics in Stockholm and conducted in accord with the Declaration of Helsinki. All subjects and one of their parents gave written informed consent.

Results

Postprandial glycaemic responses

At baseline, there was no difference in plasma glucose levels between the test meals. The postprandial glucose concentrations are shown in Fig. 1. None of the participating patients developed hypoglycaemia. The total study AUC did not differ between the test meals, but during the first two postprandial hours the AUC was larger after the low-fat compared with the high-fat meal (P = 0.047). Time-to-peak in glucose concentration was not statistically significantly different after the high-fat compared with the low-fat meal [210 min (120–240) vs. 120 min (50–240); P = 0.080].

Each subject’s glycaemic response (total study AUC) to one of the test meals was correlated with that of the other test meal (r = 0.765, P = 0.045). Analysing both tests together, a correlation was found between glycaemic response and current daily insulin dose/kg (r = 0.507, P = 0.064), but not between glycaemic response and weight, BMI or HbA1c.

There was no association between the total study AUC for glucose and the i.v. insulin infusion dose.

Gastric emptying

The paracetamol concentrations are shown in Fig. 2. The total study AUC did not differ between the test meals, but during the
first two postprandial hours the AUC was larger after the low-fat compared with the high-fat meal \( (P = 0.041) \). Time-to-peak in paracetamol concentration was not significantly delayed after the high-fat compared with the low-fat meal \( [120 \text{ min } (75–180) \text{ vs. } 60 \text{ min } (60–120); P = 0.031] \). Peak concentrations, \( C_{\text{max}} \), did not differ with statistical significance between meals. There was no statistically significant association between the total study AUC for paracetamol and the i.v. insulin infusion dose.

**Associations between postprandial glycaemic responses and gastric emptying**

After the low-fat meal, there were correlations between glucose and paracetamol concentrations at 10, 20, 30, 40, 50, 60 and 75 min \( (r = 0.530–0.788) \), and after the high-fat meal at 30, 40, 50, 60 and 120 min \( (r = 0.422–0.728) \). As analysed in the mixed model, there was a statistically significant correlation between glucose concentrations at 90 min minus that at 0 min and at 240 min minus that at 90 min and the corresponding differences in paracetamol concentrations \( (P < 0.001) \).

**Discussion**

In this study, we have demonstrated for the first time that a meal high in fat and energy reduces the initial postprandial glycaemic response and delays gastric emptying in adolescent patients with T1DM and that the glycaemic response is correlated with gastric emptying. The reduced glycaemic response is seen despite having the same preprandial plasma glucose concentration, injecting the same preprandial insulin dose, and ingesting the same amount of carbohydrate on both test occasions. Our findings are in agreement with previous findings in healthy adult subjects \[7,27\] and in patients with T2DM \[6\].

Gastric emptying of different components of a meal, as well as intragastric meal distribution, can be described thoroughly using scintigraphy \[11\]. In this study, we estimated gastric emptying using a simpler method. The paracetamol absorption method is inexpensive, easy to use and not dependent on specific equipment, a specially trained person or the administration of a radioactive isotope, and its results correlate well with scintigraphic emptying of both solid and liquid meals \[20,23\]. The absorption of paracetamol is determined by the gastric emptying rate, and serum concentrations of paracetamol correlate with the gastric emptying of liquids \[18,19\]. The method has been used previously to assess gastric emptying of solids in patients with T1DM \[21\]. The glycaemic response is not influenced by co-ingestion of paracetamol \[28\]. However, the method is not fully standardized, is time consuming and gives only an indirect estimation of gastric emptying, and pulverized paracetamol gives the meal a bitter taste. A potential problem when adding oil to a meal is that oil may ‘layer’ on top as a result of its lower density \[29\]. However, paracetamol is preferably dissolved in water, and proper mixing of it with the meal minimizes the risk that paracetamol would be selectively partitioned. The values of the parameters of paracetamol absorption obtained in this study are similar to those reported previously in healthy adults \[21,30\], indicating that the method is valid in our study population.

We did not detect any significant difference in \( C_{\text{max}} \) between the meals, which may be due to the small number of participating patients. \( C_{\text{max}} \) is considered an accurate parameter of paracetamol absorption and is reported to be higher when gastric emptying is faster \[20,30\]. However, AUC is considered to be the most reliable parameter of paracetamol absorption \[20\].

Another limitation of this study is the absence of a group of healthy adolescents, but comparison with healthy control subjects was not within the scope of this study. Given the aims of the study, the patients served as their own controls.

The association found between glycaemic response and gastric emptying may not be fully causal. Effects of the high-fat meal other than delayed gastric emptying may have contributed to the reduced glucose concentrations. Such effects may be increased levels of incretins or other gastrointestinal hormones. It is possible that the high-fat meal in our study caused a larger glucagon-like peptide-1 secretion than the low-fat meal, which may have reduced the glucagon levels more, and thereby reduced the glucose concentrations. Further studies of such hormonal changes in patients with T1DM and under the current experimental conditions are needed to fully address this.

In this study, we cannot determine if the differences found in glycaemic response and gastric emptying are due to the addition of fat \textit{per se} or to the addition of energy, since both fat and energy content differed between the meals. However, it is not possible to add fat without adding energy if the carbohydrate and protein content are to be unchanged. The important finding in this study is that the glycaemic response is affected by fat supplementation, even though the carbohydrate content of the meals is the same.

Another limitation of the present study is that the postprandial phase was studied for only 240 min. It is possible that the full effect of delayed gastric emptying on glycaemic response was not assessed. However, in practical terms this delayed effect will be affected by the administration of a new bolus dose before the following meal, since most adolescents with T1DM have another meal within 4 h, except for the evening snack.

Most subjects in this study were hyperglycaemic on arrival at the clinic. This occurred despite our attempt to optimize their glycaemic control by telephone guidance during the weeks before the investigation. This clearly demonstrates the difficulties in reaching fasting normoglycaemia in this patient group, although anxiety about the study procedures may have resulted in stress responses that may have contributed to the hyperglycaemia. One potential concern in this study is the use of an i.v. insulin infusion to obtain normoglycaemia prior to the test. However, we did not detect any statistically significant associations between the given i.v. insulin dose and the glycaemic
responses or gastric emptying, indicating that the infusion did not effect these parameters.

Postprandial normoglycaemia is essential for improving glycaemic control and decreasing the risk for long-term complications in patients with T2DM [1,31] and probably also for patients with T1DM. Thus, our findings should influence clinical practice. For a meal high in fat and energy, the action profile of the preprandial insulin dose may have to be delayed and prolonged compared with the action profile suitable for other meals. It is possible that regular insulin injected just before a high-fat meal is superior to rapid-acting insulin analogues. Even a combination of rapid-acting and regular insulin may be useful, as is a dual-wave rapid-acting insulin analogue bolus dose using CSII [4]. Other alternatives are a repeated or a postponed dose of a rapid-acting insulin analogue. The pharmacokinetics of insulin aspart is probably suitable for a low-fat meal, but in this study the dose should have been higher since our subjects generally developed hyperglycaemia after the low-fat meal. The insulin dose used in this study was based exclusively on the carbohydrate content of the meal [25], which is a common way of determining the dose of preprandial insulin. However, the finding that the reported insulin dose/kg correlated with the total study AUC for glucose suggests that we should have adjusted the prandial dose according to an estimate of the subjects’ insulin sensitivity.

Our findings of a reduced glycaemic response after a high-fat meal can also be used for adapting the food intake in patients with T1DM. To prevent nocturnal hypoglycaemia, it may be advantageous to have a postponed and prolonged glycaemic response to the bedtime snack. This may be achieved by supplementing the snack with additional fat. In situations requiring a rapid rise in the plasma glucose concentration, the intake should not be high in fat.

We conclude that a meal high in fat and energy reduces the initial glycaemic response and delays gastric emptying compared with a low-fat meal in adolescents with T1DM. The type and dose of preprandial insulin may need adjustment not only to the carbohydrate but also to the fat content of the meal in order to reach postprandial normoglycaemia.

Competing interests

P.B. has been granted research support from Sanofi Aventis and Novo Nordisk. J.A. has been paid by Novo Nordisk and Sanofi Aventis for running different educational programmes. M.L. has no competing interests to declare.

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Effects of Fat Supplementation on Postprandial GIP, GLP-1, Ghrelin, and IGFBP-1 Levels in Adolescents with Type 1 Diabetes

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Running title: GIP, GLP-1, ghrelin, and IGFBP-1 in type 1 diabetes

Key Words: adolescence, ghrelin, IGFBP-1, incretin hormones, type 1 diabetes

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Abstract

Aims: To investigate the effects of fat supplementation on the postprandial responses of GIP, GLP-1, ghrelin, and IGFBP-1 in adolescents with type 1 diabetes (T1DM).

Methods: Seven adolescents with T1DM ingested on different days in random order a high and a low-fat meal (38 and 2 g fat, respectively) with the same carbohydrate and protein content. After individual evening doses of long-acting insulin and an overnight fast, the same prandial insulin dose was given at both meals. Postprandial blood samples were taken repeatedly during four hours. Gastric emptying was estimated by the paracetamol absorption method.

Results: The postprandial increases in GIP and GLP-1 were more pronounced after the high-fat meal than after the low-fat meal (p = 0.002 and p = 0.030, respectively). Ghrelin decreased after both meals, but more after the high-fat meal (p = 0.043). The postprandial IGFBP-1 decrease did not differ between meals. A large GLP-1 response correlated with delayed gastric emptying (p = 0.029) and high fasting ghrelin levels with low postprandial glucose levels (p = 0.007) on analysing both meals together.

Conclusion: In adolescents with T1DM, the postprandial responses of GIP, GLP-1, and ghrelin are, in contrast to IGFBP-1, dependent on meal size and composition rather than on insulin levels.
Introduction

We have recently shown that a fat-rich, mixed, solid meal reduces the initial (0-120 min) glucose response and delays gastric emptying in adolescents with type 1 diabetes (T1DM) compared with a low-fat meal [1]. The delayed gastric emptying is probably responsible for some or most of the reduction in glucose concentrations as associations between gastric emptying of carbohydrates and glycaemic response have been reported, both in healthy adults and in adults with T1DM and type 2 diabetes (T2DM) [2,3]. In our study, we also found a significant correlation between gastric emptying rate and glycaemic response [1]. The effects of adding fat to mixed meals on the secretion of gastrointestinal hormones in adolescents with T1DM are not known. These gut hormones exert important effects on gastric emptying and glycaemia.

The circulating levels of the incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), increase more after a meal with a higher energy content than after a meal with a lower energy content, but the same composition of energy-providing nutrients [4]. When given their usual prandial insulin dose, adults with T1DM have similar responses to those of lean healthy controls [4]. Since insulin has been reported to inhibit GLP-1 secretion [5], the insulin dose given to patients lacking endogenous insulin secretion may influence the postprandial GLP-1 response.

The incretins stimulate glucose-dependent insulin secretion [6]. GLP-1 reduces postprandial glucose excursions also by delaying gastric emptying [7,8], also in adults with T1DM [9]. Furthermore, GLP-1 inhibits glucagon secretion and promotes satiety [6]. All these effects prevent hyperglycaemia, both fasting and postprandial, and are probably also relevant for patients with T1DM, except for the stimulation of insulin secretion [10]. In contrast to GLP-1,
GIP stimulates glucagon secretion during euglycaemia [11] and has no effect on the gastric emptying rate [12] in healthy adults. GIP promotes lipogenesis in adipose tissue [5], which may be of increased importance for adolescents with T1DM as they have higher weights and increased body fat mass compared with healthy adolescents [13-15].

In healthy adults, the postprandial ghrelin suppression is greater after a meal with a higher energy content than after a meal with a lower energy content, but the same composition of energy-proving nutrients [16]. Ghrelin and insulin levels are closely and inversely related [16,17]. Insulin is required for postprandial ghrelin suppression [18], but it is not known whether postprandial ghrelin suppression is mainly influenced by the meal-induced changes in insulin levels or by nutrient ingestion and absorption. In adolescents with new-onset T1DM, the ghrelin concentration was reported by Holdstock et al. not to decrease postprandially [19], which may influence these patients’ weight gain as ghrelin is orexigenic [20]. However, Holdstock et al. did not measure ghrelin repeatedly but only in one postprandial blood sample [19]. Therefore, they may have failed to detect a postprandial decrease in ghrelin concentration. Obese adults and patients with acromegaly, conditions associated with increased insulin resistance, lack postprandial ghrelin suppression [21,22].

Ghrelin increases plasma glucose concentrations and decreases insulin secretion acutely [23], stimulates growth hormone (GH) secretion [24], increases gastric emptying [25], stimulates appetite and food intake [20], and promotes adiposity [26]. Low fasting ghrelin values are associated with hyperinsulinaemia and insulin resistance in humans [27-29] and have been suggested to serve as a biomarker of the metabolic syndrome [30].
The GH/insulin-like growth factor I (IGF-I) axis is disturbed in adolescents with T1DM, probably due to hepatic insulinopenia leading to reduced levels of circulating IGF-I and its binding protein (IGFBP) -3 and increased levels of GH and IGFBP-1 [31]. This disturbance increases insulin resistance and may influence ghrelin levels because GH treatment of adults with GH deficiency reduces fasting ghrelin concentrations [32]. In healthy individuals, the circulating levels of IGFBP-1 fluctuate during the day and are inversely regulated by the portal supply of insulin [33], and IGFBP-1 can be used as a marker of hepatic insulization. Not much is known about the effects of meal ingestion on IGFBP-1 levels in patients lacking insulin production. In this study IGFBP-1 served as a reference protein for evaluating the possible effects of insulin on GIP, GLP-1, and ghrelin concentrations.

The main aim of the present study was to investigate whether the postprandial GIP, GLP-1, ghrelin, and IGFBP-1 responses in adolescents lacking insulin production differed between meals with different fat and energy but the same carbohydrate and protein content. We also aimed to investigate associations between these proteins and glycaemia, and between GLP-1 and ghrelin concentrations and gastric emptying.
Subjects and Methods

Patients
Nine adolescents (five girls) with T1DM for at least two years, average metabolic control and a daily insulin dose of 0.7–1.2 IU kg⁻¹ were recruited from the outpatient diabetes department at the Astrid Lindgren Children’s Hospital, Karolinska University Hospital, in Stockholm, Sweden. Exclusion criteria were coeliac or other gastrointestinal disease, major gastrointestinal symptoms, prior abdominal surgery except appendicectomy, any diabetes complication such as retinopathy, microalbuminuria and hypertension, and medication other than insulin. Two patients were excluded, one because he developed postprandial hypoglycaemia and one because he only participated once. The mean age of the remaining seven subjects was 16.4 ± 0.7 years (range: 15.5–17.5), BMI, 23.2 ± 3.4 kg/m² (range: 18.8–29.3), diabetes duration, 3.7 ± 1.2 years, HbA₁c, 7.3 ± 0.7% (Mono-S standard) and insulin dose 0.8 ± 0.2 IU kg⁻¹ day⁻¹. All were in Tanner stage 5 and treated with multiple insulin injections using basal insulin glargine once daily before dinner or at bedtime and insulin aspart or insulin lispro before every meal and snack.

Study procedure
Gastric emptying rate and postprandial glycaemic response were investigated simultaneously with this study and their methods have been described previously [1]. The subjects ingested two test meals in random order on different days separated by six to 14 days. They came to the diabetes clinic at 8 p.m. after fasting overnight and had not taken any insulin since the evening before. An intravenous (i.v.) cannula was placed in the antecubital vein of each arm. One arm was warmed by heating pads for arterilization of the blood and used for sampling for glucose and hormone analyses [34].
The subjects needed to be normoglycaemic at baseline, i.e. when ingestion of the meal started, as both hyperglycaemia and hypoglycaemia affect the gastric emptying rate [35,36]. Therefore, a variable insulin infusion (0.02–0.2 IU kg⁻¹ hour⁻¹) was given intravenously prior to baseline to subjects with hyperglycaemia (> 7.5 mmol/l). If a subject had hypoglycaemia (< 4.0 mmol/l), the test was not performed that day. Before the high-fat meal, four of the subjects received an insulin infusion, and to them 4.0 (0.3–5.8) IU was given during 37.5 (5–85) min. Before the low-fat meal, six of the subjects received an insulin infusion and, to them, 3.6 (2.5–17.3) IU was given during 42.5 (30–110) min. After the end of an insulin infusion at least 30 min passed to baseline.

At baseline, a subcutaneous injection of insulin aspart 7 IU [37] was given laterally to the umbilicus in the same place on both occasions to all subjects. Blood samples were taken before and after the insulin infusion if given, at baseline, and thereafter repeatedly during four hours. All subjects were in a sitting position during the whole study period.

Test meals
The test meals were prepared in advance by two dieticians and stored at -20°C. The meals consisted of pasta with a sauce of tomatoes and ham with or without rapeseed oil. The total energy content of the high-fat meal was 640 kcal with a fat content of 340 kcal (38 g, 53 energy per cent [E%]) and, of the low-fat meal, 320 kcal with a fat content of 20 kcal (2 g, 6 E%). Both types of meals contained the same amount of carbohydrates (240 kcal, 60 grams) and protein (60 kcal, 15 grams). The subjects were allowed to drink 100 ml of water together with the meal, which was ingested in 15 min in a sitting position. Gastric emptying was assessed by the paracetamol absorption method [38,39] with 30 mg kg⁻¹ body weight
paracetamol (Alvedon® tablet, AstraZeneca Sverige AB, Södertälje, Sweden) being pulverized in a mortar and mixed into the meals.

**Analyses**

HbA₁c was measured by an immunochemical method (Cobas Integra 400, Roche Diagnostics Scandinavia AB, Bromma, Sweden) and the Mono S standard [40] was used. HbA₁c, standardized according to the National Glycohemoglobin Standardization Program, equals 0.92 times HbA₁c measured with the Mono-S standard plus 1.33. The reference interval was < 5.2% and the coefficient of variation (CV) was < 3%. Plasma glucose concentrations during the insulin infusion were measured using bedside equipment based on a glucose dehydrogenase method (HemoCue B-Glucose Analyzer, HemoCue AB, Ängelholm, Sweden).

Baseline and postprandial plasma glucose concentrations were measured by a glucose oxidase-based method (Synchron LX20, Beckman Coulter AB, Bromma, Sweden) with the intra- and interassay CV < 4%. Serum paracetamol concentrations were measured using fluorescence polarization immunoassay technology (TDx/TDxFLx, Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA). The detection limit was 1.00 µg/ml (6.6 µmol/l) and the intra- and interassay CVs were < 5%. Incretin hormones, ghrelin, and IGFBP-1 were measured using radioimmunoassays (RIAs). Total GIP immunoreactivity was measured using the C-terminally directed antiserum R65 [41,42], which reacts fully with intact GIP and the N-terminally truncated metabolite GIP(3-42). The assay has a detection limit of 3 pmol/l and an intraassay CV of approximately 6%. GIP concentrations below the detection limit were set at 3 pmol/l in the statistical analyses. Total GLP-1 immunoreactivity was measured as described previously [43], using standards of synthetic GLP-1(7-36)amide and antiserum no. 89390. The assay cross-reacts < 0.01% with C-terminally truncated fragments and 83% with GLP-1(9-36)amide and has a detection limit of 1 pmol/l. Intra- and interassay CVs were < 6%
and 15%, respectively, at 40 pmol/l. GLP-1 concentrations below the detection limit were set at 1 pmol/l in the statistical analyses. Both GIP and GLP-1 are rapidly inactivated by the enzyme dipeptidyl peptidase-4, and the peripheral plasma concentrations of the intact hormones therefore do not reflect their rates of secretion from the gut. By measuring total hormone concentrations (both the intact hormone and the metabolite), it is possible to get a better estimate of the rates of secretion. Total ghrelin serum levels were analysed using a RIA kit from Linco Research (GHRT-89HK; Linco Research Inc., Missouri, USA) according to the manufacturer’s instructions. Intra- and interassay CVs were 3.3–10.0% and 14.7–17.8%, respectively. The detection limit was 100 pg/ml. Total IGFBP-1 serum levels were determined by a RIA described by Pihl et al. [44], and modified from Westwood et al. [45]. The detection limit was 3 ng/ml and intra- and interassay CVs were 5.6% and 11.8%, respectively. IGFBP-1 concentrations below the detection limit were set at 3 ng/ml in the statistical analyses.

Statistics and Ethics

The results are given as the mean ± S.D. or the median (range), where appropriate. The area under the curve (AUC) was calculated according to the trapezium rule. Comparisons were made with the paired samples $t$-test and the Wilcoxon signed ranks test, where appropriate. Correlations were calculated as Pearson’s correlation coefficient and Spearman’s rank correlation coefficient, where appropriate. Multiple linear regression analyses were also performed. Statistical significance was set at $p < 0.05$.

The ghrelin concentrations in our subjects did not follow a normal distribution and there was a large interindividual variation as previously reported [46]. Therefore, ghrelin was also
expressed relative to the average postprandial concentration for each subject, which made the values normally distributed.

The study was approved by the Regional Council on Ethics in Stockholm and conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. All subjects and one of their parents gave their written informed consent to participate.
Results

Glycaemic response and gastric emptying

Both the AUC 0-120 min for glucose concentrations and the AUC 0-120 min for paracetamol concentrations were smaller after the high-fat meal than after the low-fat meal (p = 0.047 and p = 0.041, respectively). Time-to-peak in paracetamol and glucose concentrations tended to be delayed after the high-fat meal (p = 0.051 and p = 0.080, respectively) [1].

GIP

The postprandial total GIP concentrations are shown in figure 1. Neither initial (i.e. at baseline or before insulin infusion, if given) nor baseline concentrations differed between meals. The concentrations increased significantly after both meals (from 3.0 [3–3] pmol/l to 73.0 [20–99] pmol/l, p = 0.018, and from 3.0 [3–7] pmol/l to 18.0 [14–34] pmol/l, p = 0.018, for high and low-fat meals, respectively). The postprandial peak value (Cmax), AUC 0-240 min and AUC 0-120 min were larger after the high-fat compared with the low-fat meal (p = 0.004, p = 0.002, and p = 0.002, respectively). Time-to-peak did not differ between meals.

GLP-1

The postprandial total GLP-1 concentrations are shown in figure 2. Neither initial nor baseline concentrations differed between meals. The concentrations increased significantly after both meals (from 14.4 ± 4.0 pmol/l to 40.4 ± 11.8 pmol/l, p < 0.001, and from 16.1 ± 6.8 pmol/l to 33.7 ± 8.2 pmol/l, p < 0.001, for high and low-fat meals, respectively). The Cmax and AUC 0-120 min were larger after the high-fat meal than after the low-fat meal (p = 0.023 and p = 0.030, respectively). Time-to-peak tended to be delayed after the low-fat meal (180 [40–210] min vs. 60 [20–240] min, p = 0.075).
Ghrelin

The postprandial relative ghrelin values are shown in figure 3. Neither the initial nor the baseline absolute ghrelin concentrations differed between meals. The absolute concentrations decreased significantly after both meals (from 605 [438–1376] pg/ml to 485 [324–1117] pg/ml, p = 0.018, and from 646 [400–1336] pg/ml to 574 [400–1082] pg/ml, p = 0.028, for high and low-fat meals, respectively). The relative decrease tended to be larger after the high-fat compared with the low-fat meal (17.1 [9.3–34.7]% vs. 13.1 [0.0–30.5]%, p = 0.063). The relative ghrelin values decreased significantly after both meals (p = 0.018 and p = 0.028 for high and low-fat meals, respectively). The AUC$_{0-240\text{ min}}$ for relative ghrelin concentrations was smaller after the high-fat meal than after the low-fat meal (p = 0.043). Time-to-reach the nadir did not differ between meals.

IGFBP-1

The pre- and postprandial total concentrations of IGFBP-1 are shown in figure 4. The initial values, which may at least partly reflect overnight hepatic insulinization, did not differ between meals (p = 0.842). In patients receiving i.v. insulin infusion (on average, 4.0 IU and 3.6 IU before high and low-fat meals, respectively) a rapid and significant decrease in IGFBP-1 concentrations was observed before meal ingestion (p = 0.037 and p = 0.036 for high and low-fat meals, respectively). The concentrations continued to decrease significantly after the standard 7 IU sc dose of insulin given at baseline (p = 0.018 for both meals). The apparent early postprandial rise in IGFBP-1 concentration after the low-fat meal was also seen in some patients after the high-fat meal and both in patients receiving and not receiving i.v. insulin. The apparent late postprandial rise in IGFBP-1 concentration after the high-fat meal was seen in one patient only. The absolute and relative decreases from the initial value, the AUC$_{0-120\text{ min}}$ and time-to-reach the nadir did not differ between meals.
Associations with the glycaemic response

The postprandial GIP, GLP-1, ghrelin, and IGFBP-1 responses did not correlate significantly with the glycaemic response (AUC 0-120 min for glucose). A higher initial ghrelin level was correlated with a lower glycaemic response (ρ = -0.684, p = 0.007) on analysing both meals together, and that association was not affected by the i.v. insulin infusion dose as analysed in a multiple linear regression model using AUC 0-120 min for glucose as outcome variable. The statistical significance for the correlation between initial ghrelin and AUC 0-120 min for glucose was lost for the high-fat meal when the meals were analysed separately (ρ = -0.643, p = 0.119 and ρ = -0.786, p = 0.036 for high and low-fat meals, respectively).

The initial IGFBP-1 concentration correlated with the initial plasma glucose concentration (r = 0.679, p = 0.008 on analysing both meals together and r = 0.588, p = 0.165, and r = 0.810, p = 0.027, for high and low-fat meals, respectively), but not with the postprandial glycaemic response.

Associations with gastric emptying

A larger early GLP-1 secretion (AUC 0-120 min for GLP-1) was associated with a slower gastric emptying rate (time-to-peak in paracetamol) (r = 0.583, p = 0.029) on analysing both meals together. That relationship was not affected by the i.v. insulin infusion dose as analysed in a multiple linear regression model using time-to-peak in paracetamol as outcome variable. The statistical significance for the correlation between AUC 0-120 min for GLP-1 and time-to-peak in paracetamol was lost when each meal was analysed separately (r = 0.582, p = 0.170 and r = 0.356, p = 0.433 for high and low-fat meals, respectively). Ghrelin concentrations did not correlate with gastric emptying parameters.
Influence of i.v. insulin infusion

In patients receiving i.v. insulin infusions, there was no significant change in GIP, GLP-1, or ghrelin concentrations before meal ingestion. The i.v. insulin infusion dose did not correlate with postprandial GIP, GLP-1, or ghrelin responses. It did correlate with the reduction in IGFBP-1 from the initial value ($\rho = 0.667, p = 0.009$) on analysing both meals together, but that correlation only reached statistical significance for the low-fat meal ($\rho = 0.630, p = 0.129$ and $\rho = 0.757, p = 0.049$ for high and low-fat meals, respectively).
Discussion

In this study we find that the GIP and the early GLP-1 responses are more pronounced after a high-fat meal than after a low-fat meal in adolescents with T1DM. This is in accord with previous findings in adults with and without T1DM ingesting meals with different energy content but the same composition [4]. For the first time, we report that ghrelin decreases after meal ingestion in adolescents with T1DM, and the suppression is more pronounced after a meal with a higher energy and fat content. A more pronounced ghrelin suppression after a meal with a higher energy content, but the same composition of energy providing nutrients, has been reported in healthy adults [16]. Given that patients lacking endogenous insulin secretion were studied, that euglycaemia was ensured before the ingestion of the meal, and that a standard prandial insulin dose was given, the differences in GIP, GLP-1, and ghrelin responses found in this study cannot be attributed to meal associated differences in insulin release. In contrast, IGFBP-1, which is predominantly insulin regulated, decreases before meal ingestion starts in patients receiving an i.v. insulin infusion and the postprandial decrease does not differ between meals even though the energy content is twice as high in the high-fat meal as in the low-fat one.

The lower early postprandial glucose concentrations found after the high-fat meal [1] may seem to be beneficial for adolescents with T1DM. However, the high fat intake also has negative effects. One potentially negative effect is the pronounced GIP response seen after the high-fat meal, given that GIP is lipogenic and adolescents with T1DM already have increased body weight and body fat mass compared with healthy peers [13-15]. On the other hand, the pronounced GIP response seen after the high-fat meal may not be due to the large fat content per se, but to the large energy content of that meal, since similar GIP responses to isocaloric
meals consisting of either carbohydrates or fat has been found previously in healthy young adults [47].

The difference in the GLP-1 response between the meals in this study is related to the timing of the increase rather than to the total quantity of hormone secreted. The high-fat meal gives rise to a larger early GLP-1 response than the low-fat meal. Accordingly, we find an association between a larger early GLP-1 response and a delay in gastric emptying. The limited number of patients studied here does not allow us to establish this finding separately for each meal. This association between endogenous GLP-1 secretion and gastric emptying has not been reported previously in patients with T1DM, but it is in accord with the effect of exogenous GLP-1 given to healthy adults and patients with T1DM and T2DM [5,7]. A larger early GLP-1 response after the high-fat meal may be important for the delay in gastric emptying seen after such a meal and should, as we have reported in this patient group [1], lead to an attenuated initial glycaemic response. However, a direct association between GLP-1 and glycaemic response was not detected.

One advantage of the current study design in T1DM patients is that, after ensuring euglycaemia, the same prandial insulin dose was administered to each subject. Therefore, we can conclude that the possible insulin feedback on GLP-1 does not play a major role in determining the GLP-1 response to a meal. At least in comparison with IGFBP-1, GLP-1 secretion is not very sensitive to insulin, and other mechanisms are important for the different GLP-1 responses observed after meals with different fat and energy contents.

The finding in this study of a postprandial decrease in ghrelin concentrations is in accord with findings in healthy adults [17], but contradicts a report by Holdstock et al. investigating
adolescents with T1DM [19]. It is possible that Holdstock et al. failed to observe a fall by only determining ghrelin at one postprandial time point. It is also possible that differences in duration of diabetes between the study populations may have affected the results. Nevertheless, the postprandial ghrelin suppression found in our study may be both smaller and delayed compared with the suppression found in healthy adults [16,17]. This needs to be clarified in future controlled studies. Subnormal postprandial ghrelin suppression in adolescents with T1DM may be due to their increased insulin resistance or their elevated GH levels since obese adults and patients with acromegaly lack postprandial ghrelin suppression [21,22]. If subnormal postprandial ghrelin suppression is confirmed in adolescents with T1DM, it may be one cause of their increased weight.

The association found in this study between high fasting ghrelin concentrations and low postprandial glucose concentrations supports previous reports that ghrelin is associated with insulin sensitivity. In obese and insulin-resistant adults, fasting ghrelin concentrations are suppressed [27-29]. Since all subjects in our study had similar baseline glucose levels, ingested the same amount of carbohydrates, were given the same prandial insulin dose, not adjusted for individual insulin sensitivity, and their postprandial glycaemic responses differed largely and consistently after both meals (data not shown), a larger postprandial glucose increase probably reflects higher insulin resistance, which is associated in our study with a lower fasting ghrelin level. Future studies should confirm that fasting ghrelin levels are determined by insulin sensitivity in adolescents with T1DM and investigate the involved mechanisms.

A higher fasting IGFBP-1 concentration is associated in this study with a higher fasting plasma glucose concentration. A similar association is not found for postprandial glucose.
concentrations, indicating that hepatic insulinization, of which IGFBP-1 is a marker, is more important for fasting than for postprandial glycaemia. For postprandial glycaemia, peripheral insulin sensitivity may be of greater importance.

One of the limitations of the current study is the use of an i.v. insulin infusion prior to meal ingestion. But gastric emptying, which influences postprandial glycaemia and potentially hormonal responses to meals, is significantly affected by preprandial glucose levels [35,36]. Therefore, it is essential to obtain normoglycaemia at baseline. Overnight euglycaemic clamps would have been preferred, but were too costly. However, GIP, GLP-1, and ghrelin do not show any acute changes in response to i.v. insulin infusion or any delayed effects influencing postprandial responses. In contrast, we find that the i.v. insulin dose affects IGFBP-1, a protein known to be regulated by insulin.

We conclude that GIP, GLP-1, and ghrelin responses differ between a high and a low-fat meal in adolescents with T1DM even though they are given the same prandial insulin dose at both meals. A large postprandial GLP-1 response is associated with slow gastric emptying. High fasting ghrelin levels are associated with low postprandial glycaemic levels in adolescents with T1DM, which may be due to ghrelin’s association with insulin sensitivity.
Acknowledgements

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Figure 1. Total GIP concentrations (pmol/l, means ± S.D.) after a high-fat (black triangles) and a low-fat meal (white circles) in seven adolescents with type 1 diabetes. $C_{\text{max}}$, $\text{AUC}_{0-240\text{ min}}$, and $\text{AUC}_{0-120\text{ min}}$ were larger after the high-fat meal ($p = 0.004$, $p = 0.002$, and $p = 0.002$, respectively).
Figure 2. Total GLP-1 concentrations (pmol/l, means ± S.D.) after a high-fat (black triangles) and a low-fat meal (white circles) in seven adolescents with type 1 diabetes. $C_{\text{max}}$ and AUC $>_0\text{min}$ were larger after the high-fat meal ($p = 0.023$ and $p = 0.030$, respectively). Time-to-peak tended to be delayed after the low-fat meal ($p = 0.075$).
Figure 3. Relative ghrelin values (means ± S.D.) after a high-fat (black triangles) and a low-fat meal (white circles) in seven adolescents with type 1 diabetes. The relative ghrelin value was calculated as outlined in methods. The relative ghrelin values decreased significantly after both meals (p = 0.018 and p = 0.028 for high and low-fat meals, respectively). The AUC 0-240 min for relative ghrelin was smaller after the high-fat meal than after the low-fat meal (p = 0.043).
**Figure 4.** Pre- and postprandial concentrations of total IGFBP-1 (ng/ml, means ± S.D.) in seven adolescents with type 1 diabetes eating a high-fat meal (black triangles) and a low-fat meal (white circles) on separate days. Insulin was given intravenously to subjects with hyperglycaemia before initiating meal ingestion. At time 0 min, when ingestion started, all subjects were normoglycaemic and given 7 IU insulin aspart sc.
References


Higher drive for thinness in adolescent males with insulin-dependent diabetes mellitus compared with healthy controls

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Eating behaviour in adolescent males with insulin-dependent diabetes mellitus (IDDM) living in central Sweden was compared with that of healthy age-matched male controls using the Eating Disorder Inventory for Children and an interview. The patients were heavier than controls \( (p = 0.004) \) and had higher Drive for Thinness scores \( (p = 0.002) \). None was diagnosed as having a current eating disorder.

**Conclusion**: The results of the study may indicate an increased risk of future eating disorders in males with IDDM.

**Key words**: Adolescents, diabetes mellitus, eating disorder, males, metabolic control

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Studies of eating disorders in patients with insulin-dependent diabetes mellitus (IDDM) have hitherto focused mainly on females. In the general population, males account for 5–10% of patients with anorexia nervosa (1) and 10–15% of patients with bulimia nervosa (2). In a North American study, 2% of adolescent males were found to have a disordered eating pattern (3). The proportion of males seeking medical advice and treatment for eating disorders has increased in the USA (4). Studies have shown that the differences in eating disorders between males and females concerning psychopathology and comorbidity are less pronounced than the similarities (4).

Dieting and weight loss, which are social demands for women, are considered as risk factors for developing eating disorders. Braun et al. suggest that for males a hobby or sport that focuses on body weight and performance might be a risk factor for eating disorders (4). Diabetes mellitus has also been proposed as such a risk factor (5). Insulin omission or underdosing offers an effective but dangerous means of losing weight in patients with IDDM. Insulin omission deteriorates metabolic control, and Rydall et al. found a strong association between disordered eating behaviour, poor metabolic control and a greater risk of developing retinopathy (6).

This study investigated eating behaviour in adolescent males with IDDM and compared it with that in age-matched healthy male control subjects.

All males, 14–18 y of age, who had had IDDM for more than 1 y and were living in 5 counties of central Sweden (Örebro, Dalarna, Värmland, Södermanland and Västmanland) \( (n = 141) \), were identified. Two of them were not included as they were not scheduled in time. The patients were examined during an ordinary visit to the clinic. A protocol was filled in by the physician or diabetes nurse regarding height, weight, social factors, type of insulin regimen and presence of any diabetes complications. Glycosylated haemoglobin (HbA1c) was analysed using high-performance liquid chromatography. The mono S standard was used, which gives on average 1% lower values than the DCCT standard (7). The reference interval for healthy people is 3.5–5.3%.

As a control group, 141 age-matched males without IDDM were identified from school records of 2 schools in Örebro. Each control subject was chosen as the one who was born closest to each index person. Their weight and height were measured at the office of the school nurse. They filled in a form concerning social factors and presence of any illness. Two of the chosen controls did not attend for the examinations.

To assess eating behaviour, all participating patients and control subjects anonymously filled in a self-administered questionnaire concerning eating attitudes and psychopathological factors associated with eating disorders, the Eating Disorder Inventory for Children (EDI-C) (8). The response rate among the patients was...
The patients also answered anonymously a few questions concerning their diabetes treatment and whether they had ever taken less insulin than they should in order to lose weight.

In the second stage, patients and control subjects scoring 14 or above on the Drive for Thinness subscale in EDI-C were examined by a child and adolescent psychiatrist (IE) who conducted a semi-structured interview called RAB-T. This is a version of Rating of Anorexia and Bulimia (RAB) (9) adjusted for teenagers. Patients reporting insulin omission with the aim of losing weight were also interviewed. The purpose of the interviews was to determine whether or not the subject had an eating disorder according to the DSM-IV criteria, and if so, of what type.

The mean age and height did not differ between patients and control subjects, but the mean weight and body mass index (BMI) were significantly higher in the group of patients (Table 1). Non-participating patients had a higher mean HbA1c than those who participated and that difference might influence the prevalence of disturbed eating since eating disorders are associated with poor metabolic control (6). No other differences were observed between the participating and non-participating patients. There was no difference in the parents’ occupations between the participating and non-participating patients and control subjects.

The Statistical Package for the Social Sciences (SPSS) was used for all analyses. Individuals lacking two or more items in a subscale of EDI-C were not included in the analysis of that subscale; for this reason, the sample sizes for specific subscales vary. The distribution of the EDI-C data was heavily skewed; therefore, the results are presented as medians and the comparisons between the groups were made with a two-tailed non-parametric test, the Mann–Whitney U-test.

The results of EDI-C are also presented as means, to allow comparisons with other studies, most of which use means. Statistical significance was set at p < 0.05.

The ethics committee of Örebro County Council approved the study.

The patients showed significantly higher scores on the Drive for Thinness subscale in EDI-C compared with the control subjects (p = 0.002) (Table 2). This finding is consistent with the hypothesis that the disease-specific focus on food and eating pattern may have been a factor in their diabetes treatment decisions.

### Table 1. Characteristics of patients with insulin-dependent diabetes mellitus (IDDM) and controls.

<table>
<thead>
<tr>
<th></th>
<th>Participating IDDM (n = 109)</th>
<th>Controls (n = 139)</th>
<th>Non-participating IDDM (n = 30)</th>
<th>p-Values (all patients vs controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>16.6 ± 1.1</td>
<td>16.4 ± 1.1</td>
<td>16.4 ± 1.2</td>
<td>ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.8 ± 12.1</td>
<td>66.7 ± 11.0</td>
<td>70.5 ± 12.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.3 ± 7.8</td>
<td>175.6 ± 7.3</td>
<td>175.4 ± 8.2</td>
<td>ns</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>22.4 ± 3.1</td>
<td>21.6 ± 3.1</td>
<td>22.9 ± 3.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Living with both parents (%)</td>
<td>66</td>
<td>77</td>
<td>53</td>
<td>0.016</td>
</tr>
<tr>
<td>Duration of diabetes (y)</td>
<td>7.2 ± 4.0</td>
<td></td>
<td>7.9 ± 4.5</td>
<td>ns⁺</td>
</tr>
<tr>
<td>Daily insulin dosage (U kg⁻¹)</td>
<td>1.0 ± 0.3</td>
<td></td>
<td>1.0 ± 0.3</td>
<td>ns⁺</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.6 ± 1.5</td>
<td></td>
<td>8.5 ± 1.9</td>
<td>0.007⁺</td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise stated.

⁺ Comparison between participating and non-participating patients.

BMI: body mass index; HbA1c: major fraction of glycosylated haemoglobin; ns: not significant.

### Table 2. Results of the Eating Disorder Inventory for Children (EDI-C) questionnaire in patients with insulin-dependent diabetes mellitus (IDDM) and controls.

<table>
<thead>
<tr>
<th>Subscale</th>
<th>IDDM patients</th>
<th>Controls</th>
<th>n</th>
<th>Median⁴</th>
<th>Mean</th>
<th>Min-max</th>
<th>n</th>
<th>Median⁴</th>
<th>Mean</th>
<th>Min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drive for Thinness†</td>
<td>109</td>
<td>0.0 (0.0–1.0)</td>
<td>1.18</td>
<td>0–21</td>
<td>139</td>
<td>0.0 (0.0–0.0)</td>
<td>0.53</td>
<td>0–15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulimia‡</td>
<td>108</td>
<td>0.0 (0.0–1.0)</td>
<td>1.14</td>
<td>0–21</td>
<td>138</td>
<td>1.0 (0.0–3.0)</td>
<td>1.48</td>
<td>0–8</td>
<td></td>
<td></td>
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<tr>
<td>Body Dissatisfaction</td>
<td>109</td>
<td>2.0 (0.0–4.0)</td>
<td>3.05</td>
<td>0–22</td>
<td>139</td>
<td>1.0 (0.0–3.0)</td>
<td>2.38</td>
<td>0–19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ineffectiveness</td>
<td>108</td>
<td>0.0 (0.0–1.0)</td>
<td>0.88</td>
<td>0–8</td>
<td>138</td>
<td>0.0 (0.0–1.0)</td>
<td>0.81</td>
<td>0–12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfectionism</td>
<td>109</td>
<td>2.0 (1.0–3.0)</td>
<td>2.85</td>
<td>0–18</td>
<td>137</td>
<td>3.0 (1.0–4.0)</td>
<td>3.03</td>
<td>0–13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interpersonal Distrust</td>
<td>109</td>
<td>3.0 (1.0–5.0)</td>
<td>3.13</td>
<td>0–10</td>
<td>139</td>
<td>2.0 (1.0–3.0)</td>
<td>2.45</td>
<td>0–11</td>
<td></td>
<td></td>
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<tr>
<td>Interoceptive Awareness</td>
<td>107</td>
<td>0.0 (0.0–2.0)</td>
<td>1.45</td>
<td>0–22</td>
<td>138</td>
<td>0.0 (0.0–2.0)</td>
<td>1.14</td>
<td>0–8</td>
<td></td>
<td></td>
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<tr>
<td>Maturity Fears</td>
<td>108</td>
<td>5.0 (3.0–8.0)</td>
<td>5.75</td>
<td>0–17</td>
<td>138</td>
<td>5.0 (3.0–7.0)</td>
<td>5.71</td>
<td>0–19</td>
<td></td>
<td></td>
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<tr>
<td>Asceticism</td>
<td>109</td>
<td>6.0 (4.0–8.0)</td>
<td>6.07</td>
<td>0–14</td>
<td>139</td>
<td>6.0 (3.0–8.0)</td>
<td>6.03</td>
<td>0–14</td>
<td></td>
<td></td>
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<tr>
<td>Impulse Regulation</td>
<td>109</td>
<td>0.0 (0.0–2.0)</td>
<td>1.54</td>
<td>0–16</td>
<td>139</td>
<td>0.0 (0.0–3.0)</td>
<td>1.89</td>
<td>0–12</td>
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<tr>
<td>Social Insecurity</td>
<td>108</td>
<td>1.0 (0.0–2.0)</td>
<td>1.60</td>
<td>0–8</td>
<td>139</td>
<td>1.0 (0.0–3.0)</td>
<td>1.87</td>
<td>0–18</td>
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<td></td>
</tr>
</tbody>
</table>

⁴ Median (25th–75th percentile).

† Significantly higher value in IDDM group on the Drive for Thinness subscale (p = 0.002).

‡ Significantly higher value in controls on the Bulimia subscale (p = 0.01).
contribute to disturbed eating in this age group. The higher weight found among patients may also produce a stronger desire to lose weight. Two patients and one control subject scored above 14 on the Drive for Thinness subscale. On the Bulimia subscale, the control group had significantly higher scores than the IDDM group \((p = 0.01)\). Those with high scores on the Bulimia subscale did not differ in any other respect from those with lower bulimic scores. Meltzer et al. also found more symptoms of bulimia in the male control group than in the male IDDM group \((10)\), indicating that bulimic symptoms may, to some extent, be normal behaviour among healthy adolescent males, whereas patients with IDDM may have to suppress bulimic behaviour.

There was no significant difference between the two groups on any other EDI-C subscale, indicating that male adolescents with IDDM cope quite well with their situation and that their mental status is not inferior to that of their healthy peers.

Three patients admitted to omitting insulin in the questionnaire, but all of them denied it in the interview. One of them also had a high score on the Drive for Thinness subscale.

Twenty-one patients reported that they had forgotten one dose of insulin at least twice a month. These patients had a higher mean HbA\(_1c\) than those who had not forgotten their insulin \((8.3 \pm 1.9\% \; vs \; 7.4 \pm 1.3\%, \; p = 0.045)\), but were not distinguished from the others in any other way. This finding corresponds well to recent results by Morris et al. \((11)\), who reported insulin underdosing as a major explanation for poor metabolic control in adolescents with IDDM.

Five subjects went on to the second stage of the study. The result of the interview showed that none of them fulfilled the DSM-IV diagnostic criteria for a current eating disorder. One of them may have had an eating disorder that fulfills the DSM-IV diagnostic criteria for a current eating disorder. One of them may have had an eating disorder. The reason for this association is unknown, but corresponds well with the findings of Forsander et al. \((12)\).

In some studies overweight has been found more frequently before the onset of an eating disorder in males than in females \((14, 15)\).

To the authors’ knowledge, this sample size is the largest ever studied concerning the association between IDDM and eating disorders among adolescent males using a two-step procedure, but it is still small, considering the low prevalence of eating disorders among males in the general population. Therefore, no conclusions may be drawn as to whether or not there is an increased frequency of eating disorders among IDDM males. The finding of significantly higher scores on the Drive for Thinness subscale among patients compared with control subjects may indicate a higher risk for developing a disordered eating in the future, but the scores were still very low for both groups. A study with a prospective design is needed to answer the question of increased risk and frequency of eating disorders among adolescent males with IDDM.

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