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 insulinotropic 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 controls1

1 My surname was Svensson before I married in July 2003.
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<tbody>
<tr>
<td>AN</td>
<td>Anorexia nervosa</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BED</td>
<td>Binge eating disorder</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BN</td>
<td>Bulimia nervosa</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>$C_{\text{max}}$</td>
<td>Maximal concentration</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CSII</td>
<td>Continuous subcutaneous insulin infusion</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DPP-IV</td>
<td>Dipeptidyl peptidase IV</td>
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<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<tr>
<td>E%</td>
<td>Energy per cent</td>
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<tr>
<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>
</tr>
<tr>
<td>EDNOS</td>
<td>Eating disorder not otherwise specified</td>
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<tr>
<td>EGG</td>
<td>Electrogastrography</td>
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<td>ENS</td>
<td>Enteric nervous system</td>
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<td>FFQ</td>
<td>Food frequency questionnaire</td>
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<tr>
<td>GH</td>
<td>Growth hormone</td>
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<tr>
<td>GHRH</td>
<td>Growth hormone releasing hormone</td>
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<td>GHS</td>
<td>Growth hormone secretagogue</td>
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<tr>
<td>GHS-R</td>
<td>Growth hormone secretagogue receptor</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic polypeptide</td>
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<td>GIPR</td>
<td>Glucose-dependent insulinotropic polypeptide receptor</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide 1</td>
</tr>
<tr>
<td>GLP-1R</td>
<td>Glucagon-like peptide 1 receptor</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>ICC</td>
<td>Interstitial cells of Cajal</td>
</tr>
<tr>
<td>IGFBP</td>
<td>Insulin-like growth factor binding-protein</td>
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<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td>ISPAD</td>
<td>International Society for Pediatric and Adolescent Diabetes</td>
</tr>
<tr>
<td>IU</td>
<td>International units</td>
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<tr>
<td>Iv</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>MDI</td>
<td>Multiple daily injections</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<tr>
<td>Sc</td>
<td>Subcutaneous</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>SDS</td>
<td>Standard deviation score</td>
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<tr>
<td>Acronym</td>
<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>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</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, \( \gamma \)-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 insulinotropic 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-cells [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)NH$_2$ 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-Gln\(^{14}\)-ghrelin, and obestatin are all produced from preproghrelin. Des- Gln\(^{14}\)-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 adrenocorticotropic 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 in vitro studies [161], and promotes adiposity in a diet-depending 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 co morbidity 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>Patients</th>
<th>Controls</th>
<th>Patients</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Studies I and II</td>
<td>Study Ia</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>

a 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 outpatient 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 $\geq 14$ 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”, “Interoceptive Awareness”, “Maturity Fears”, “Asceticism”, “Impulse Regulation”, and “Social Insecurity”.

28
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 soft-ware 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></th>
<th>Boys N = 16</th>
<th>Girls N = 22</th>
<th>SNR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ISPAD&lt;sup&gt;b&lt;/sup&gt;</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></td>
<td>&lt; 10</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>Polysaturated 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&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fibre (g/MJ)</td>
<td>2.2 (0.9)</td>
<td>2.2 (0.5)</td>
<td></td>
<td>2.8–3.4&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Swedish Nutritional Recommendations [267]. <sup>b</sup> International Society for Pediatric and Adolescent Diabetes [229]. <sup>c</sup> Recommendation for adults. <sup>d</sup> Calculated recommendation for the age group studied. <sup>e</sup> 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 ≥ 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 =
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_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\text{–}240} 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\text{–}120} min, the AUC_{0\text{–}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 (\( p < 0.001 \)).
4.3.8 GLP-1 response and gastric emptying

A larger early GLP-1 secretion (AUC $0-120\text{ min}$ 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 $0-120\text{ min}$ 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 $0-120\text{ min}$ for glucose). A higher initial ghrelin level was associated with a lower glycaemic response ($\rho = -0.684, p = 0.007$) 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 $0\text{-}120\text{ min}$ for glucose as outcome variable. The statistical significance for the correlation between initial ghrelin and AUC $0\text{-}120\text{ min}$ 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 $0\text{-}240\text{ min}$ for glucose or AUC $0\text{-}240\text{ min}$ 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 $80 \pm 28\%$ 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.
analogues. 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 underhudsfat 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 blodsockerkontrolen.

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 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 ofta symtom från magen jämfört med friska vuxna. En anledning till detta kan vara störningar i magtarmkanalens rörelser antingen till följd av akuta blodsockerförändringar eller kroniska skador i tarmen pga långvarigt förhöjd blodsockernivå. Tidegare har man inte säkert vetat om även ungdomar med T1DM har mer symtom från magen än friska jämnåriga.

Den psykiatriska diagnosen ätstörning samt de mildare formerna av stört åtbeteende är vanligare hos tonårsflickor och unga kvinnor med T1DM jämfört med friska kvinnliga jämnåriga. T1DM anses vara en riskfaktor för att utveckla ätstörning pga att sjukdomen och dess behandling kan ge övervikt och pga fokuseringen på vad patienten bör äta. Patienter med T1DM och ätstörning har sämre blodsockerkontroll och ökad risk för diabeteskomplikationer än patienter utan ätstörning. Förekomsten av ätstörning 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 kostrekommendationer ganska bra, men intaget av måttet fett är högre och intaget av fiber är lägre än rekommenderat. Patienter som har lång tid med blodsockerkontroll åter mer fett och mindre kolhydrater än patienter som har lång tid med 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 cigarettrökning, 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. Magtarmsymtom hos ungdomar med T1DM bör utredas och behandlas förutsätningslöst, precis som hos andra personer, och inte antas bero på diabetes sjukdomen. Men ungdomar som haft diabetes i många år, som har dålig blodsockerkontroll och symtom från övre delen av magtarmkanalen bör utredas avsände magsäckstömningshastighet.

En måltid innehållande mycket fett och kalorier ger lägre blodsocker de första två timmarna efter måltiden och långsammare magsäckstömnings hos ungdomar med T1DM jämfört med en fettsnål måltid med lägre kaloriinnehåll. Blodsockernivån efter måltid har starkt samband med magsäckstömningshastigheten. Insulindosen till en måltid bör anpassas, inte bara efter dess kolhydratinnehåll, utan även efter dess fett- och kaloriinnehåll, för att normalt blodsocker ska uppnås 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 kaloriinnehåll i en måltid kan verka gynnsamt eftersom blodsockeret då ligger lägre initialt efter måltiden, men är negativt av andra anledningar. En fett- och kaloriirik måltid ger ett kraftigt GIP-svar, vilket kan öka fettansamlingen i kroppen. Ghrelinvaret efter måltid 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.

Tonårsåldrar med T1DM rapporterar högre grad av viktfobi och väger mer än tonårsåldrarna utan diabetes. Däremot rapporterar de inte mer psykologiska avviksel associerade till ätstörningar. Tonårsåldrar med T1DM kan ha en ökad risk för att utveckla ätstörning och det bör undersökas närmare i större studier.
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