Body Composition in Adolescents with Type 1 Diabetes

Aspects of Glycaemic Control and Insulin Sensitivity

BY

STEFAN SÄRNBLAD

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Abstract

Excessive weight gain has frequently been reported in adolescents with type 1 diabetes, especially in girls. Puberty is associated with reduced insulin sensitivity that is further diminished by overweight. The causes and consequences of excessive weight gain in adolescents with type 1 diabetes are not fully understood. This thesis investigated body composition in adolescents with type 1 diabetes and the relationships between physical activity, energy intake and changes in body composition. Furthermore, the effect of metformin as additional therapy on glycaemic control and insulin sensitivity was examined in a randomised placebo-controlled study. Body mass index (BMI) and percentage body fat (%BF) was significantly higher in girls with type 1 diabetes compared to healthy control girls. Mean HbA1c during puberty, but not mean insulin dose, was positively related to BMI at the age of 18 in girls with diabetes. A centralised fat distribution was associated with poor glycaemic control, elevated daily dosage of insulin and elevated cholesterol and triglyceride levels. Neither total physical activity nor total energy intake differed between adolescent girls with type 1 diabetes and healthy age-matched control girls. A high dietary fat intake was positively related to gain in %BF in girls with type 1 diabetes. Additional therapy with metformin for three months improved glycaemic control and peripheral insulin sensitivity in adolescents with poorly controlled type 1 diabetes. The improvement in glycaemic control was related to insulin sensitivity at baseline implicating that the most insulin resistant subjects benefitted most from the metformin therapy. It is concluded that the excessive weight gain observed in girls with type 1 diabetes is mainly mediated by an increased fat mass and that dietary fat intake is of importance for this gain in body fat. Additional treatment with metformin improves glycaemic control in adolescents with poorly controlled type 1 diabetes.

Keywords: Type 1 diabetes mellitus, Adolescent, Body composition, Physical activity, Energy intake, Metformin, Insulin sensitivity

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To Elisabet, Johannes, Andreas and Sara
List of Papers

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


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### Abbreviations and definitions

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<thead>
<tr>
<th>Term</th>
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<tbody>
<tr>
<td>Adolescence</td>
<td>The period between 10 and 20 years of age, from the beginning of the development of secondary sex characteristics to cessation of growth</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FM</td>
<td>Total fat mass</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>HbA₁c</td>
<td>Haemoglobin A₁c</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor-I</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein 1</td>
</tr>
<tr>
<td>IRMA</td>
<td>Immunoradiometric assay</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>M</td>
<td>Total amount of glucose metabolised during steady state of the clamp</td>
</tr>
<tr>
<td>M/I</td>
<td>Total amount of glucose metabolised during steady state of the clamp divided by the mean insulin concentration during steady state</td>
</tr>
<tr>
<td>MIT</td>
<td>Multiple insulin injection therapy</td>
</tr>
<tr>
<td>PCO</td>
<td>Polycystic ovarian syndrome</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>%BF</td>
<td>Percentage body fat</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>TEE</td>
<td>Total energy expenditure</td>
</tr>
<tr>
<td>TNF-</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>UKPDS</td>
<td>UK Prospective Diabetes Study</td>
</tr>
</tbody>
</table>
Introduction

In the beginning of the insulin era a short stature was common in children with type 1 diabetes mellitus. Today the final height and pubertal development are considered to be comparable with those of healthy children. However, adolescents with type 1 diabetes often gain excessive weight during puberty, a phenomenon that is most common in girls. Intensification of insulin therapy has been associated with this undesired weight gain, but otherwise the reasons are not fully understood.

Insulin sensitivity is normally reduced during puberty and an elevated body mass index (BMI) further impairs the sensitivity to insulin. An elevated BMI has also been associated with poorer quality of life and with deteriorated glycaemic control. Furthermore, it is possible that excessive weight gain might trigger an eating disorder and insulin omission in an attempt to reduce weight, but often at the price of impaired metabolic control.

There is evidence that glycaemic control is linked to future microvascular complications, coronary atheromatosis and quality of life. It is thus of great importance to investigate the mechanisms underlying the excessive weight gain and to develop treatment strategies with the aim of preventing undesired weight gain and hence of improving glycaemic control and the quality of life.

Growth

Regulation of growth

Growth can be divided into four different stages according to the infancy-childhood-puberty (ICP) model, namely fetal, infancy, childhood and pubertal. The stages are regulated by different factors. Fetal and early postnatal growth is mainly determined by nutrition, although influence by the growth hormone/insulin-like growth factor (GH/IGF-I) axis has also been suggested. An indirect indication that genetic factors are of lesser important during fetal growth is the poor association between birth height and parental height. During childhood this association becomes stronger and
the correlation between height at 2 years of age and midparental height is 0.7-0.8.  

During the end of the first year of life, growth hormone begins to play a major role in the growth of the infant. The subsequent childhood growth phase is influenced not only by the GH/IGF-I axis but also by thyroxine levels.  

With the initiation of puberty, the adolescent growth spurt begins. Final height is approximately 12 cm higher in boys due to an earlier pubertal start in girls and a lower peak height velocity in girls than in boys. The accelerated growth during puberty is associated with increased activity in the GH/IGF-I axis, with the maximum GH secretion occurring during mid-puberty. Sex steroids, especially oestrogens (in boys converted from testosterone), have an important role in pubertal growth by regulating GH secretion and by acting directly on the growth plate. In addition, adequate levels of thyroid hormone are important for the progress of pubertal growth.

Growth during puberty in type 1 diabetes

Height  

In most previous studies children with type 1 diabetes have been found to be taller at diagnosis than healthy controls, although this has not been confirmed in some other studies. There have been indications that this height difference is most evident among children diagnosed with diabetes during prepubertal years. Early twin studies showed that the growth velocity was decreased before the diagnosis of diabetes, although in a more recent study it was found that rapid linear growth is a risk factor for type 1 diabetes.  

Growth during puberty is frequently reported to be impaired in type 1 diabetes, with a reduced peak height velocity. There is a marked sex difference, with greater impairment in girls, especially concerning peak height velocity. The reduction of growth velocity has been related to poor metabolic control in some studies, although others have not shown any association with glycaemic control. There have been reports of normal, reduced and even increased final height in patients with type 1 diabetes. Twin studies have shown that final height is reduced in children diagnosed with type 1 diabetes before puberty. In more recent studies final height has been found to be within normal limits or even higher than target height. Not surprisingly there have been reports of a sex difference, with normal final height in boys but reduced final height in girls.  

The impaired pubertal growth might be explained by disturbances in the axis between GH and IGF-I, with low levels of free IGF-I. Poor metabolic
control is associated with low levels of IGF-I, which could explain the association between HbA1c and growth.

**BMI**

Early studies of weight in children with type 1 diabetes showed no difference compared to that in healthy children. During the last 10 years there have been several reports of increased BMI in adolescents with type 1 diabetes. This overweight has mainly been observed in girls but it has also been found in boys. The weight difference compared to controls is usually most pronounced during late stages of puberty.

Genetic factors are of importance for the development of overweight during childhood. Interestingly, Holl et al. found a positive relation between mid-parental weight and weight in boys with type 1 diabetes, but not in diabetic girls.

**Body composition during puberty**

**Healthy adolescents**

Total fat mass (FM) and lean body mass (LBM) show little differences between the sexes during the prepubertal period. During puberty total fat mass increases in both sexes. However, because of a markedly lower gain in LBM in girls, only girls show an increase in percentage body fat (%BF). At the end of puberty the proportion of body fat in girls has reached 20-25 % compared to approximately 10% in boys. The sex difference in the change of LBM is caused by differences in androgen levels. However, differences in physical activity might also be an important mediator.

**Adolescents with type 1 diabetes**

As already mentioned, most previous studies have shown that adolescents with type 1 diabetes have a higher BMI than healthy controls, with the inference that this reflects excessive fat accumulation. Body composition has, however, been less frequently studied in adolescents with type 1 diabetes. From the present data it seems that girls with diabetes have higher %BF than healthy control girls (Table 1). In all studies on body composition except one, skinfold measurements were used, and in the exception measurements were made by bioelectrical impedance. In contrast to the skinfold studies the latter study showed higher LBM in adolescents with type 1 diabetes, without any sex difference.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Number</th>
<th>Age (y)</th>
<th>Controls</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abusrewil 1989</td>
<td>58 boys 48 girls</td>
<td>UC 5-22</td>
<td>-</td>
<td>Skinfold</td>
<td>Weight = Skinfold</td>
</tr>
<tr>
<td>Gregory 1992</td>
<td>35 boys 33 girls</td>
<td>UC 6.3-18.0</td>
<td>-</td>
<td>Skinfold</td>
<td>BI &gt; %BF than</td>
</tr>
<tr>
<td>Tillmann 1995</td>
<td>36 boys 26 girls</td>
<td>CS 10-17</td>
<td>33 boys 47 girls</td>
<td>Skinfold</td>
<td>BMI &gt; triceps and biceps</td>
</tr>
<tr>
<td>Pietiläinen 1995</td>
<td>48 girls</td>
<td>CS 10-19</td>
<td>48 girls</td>
<td>Skinfold</td>
<td>&gt; %BF</td>
</tr>
<tr>
<td>Nuoffer 1996</td>
<td>43 girls</td>
<td>CS 10-20.7</td>
<td>43 girls</td>
<td>Skinfold</td>
<td>&gt; %BF</td>
</tr>
<tr>
<td>Tuvemo 1997</td>
<td>34 children</td>
<td>CS 7-18</td>
<td>5 boys 10 girls</td>
<td>Skinfold</td>
<td>BMI &gt; triceps, subscapular</td>
</tr>
<tr>
<td>Bartz 1997</td>
<td>157 boys 117 girls</td>
<td>CS 6.5-30.9</td>
<td>16 boys 17 girls</td>
<td>BI</td>
<td>&gt; LBM</td>
</tr>
<tr>
<td>Ahmed 2001</td>
<td>23 boys 23 girls</td>
<td>Long. 8-17</td>
<td>20 boys 20 girls</td>
<td>Skinfold</td>
<td>&gt; gain FM</td>
</tr>
<tr>
<td>Riihimaa 2002</td>
<td>49 boys 51 girls</td>
<td>CS 9-18</td>
<td>49 boys 51 girls</td>
<td>Skinfold</td>
<td>&gt; %BF only in Tanner V</td>
</tr>
</tbody>
</table>

UC: uncontrolled; CS: cross-sectional with healthy controls; Long.: longitudinal with healthy controls; BI: bioelectrical impedance; =: no significant difference between diabetic patients and controls; >: higher value in diabetic patients than in controls.
Energy balance

Energy balance is achieved if energy intake is equal to energy expenditure. Any imbalance, other than energy deposition for growth (≈ 84 kJ/d or 20 kcal/d), causes changes in body composition. The average energy imbalance responsible for fat gain in children is generally very small (30 kcal/d).54

Energy output or expenditure is highly variable between individuals. Total daily energy expenditure (TEE) can range from 2000 kcal in a sedentary man to 7000 kcal in a person performing heavy labour. Approximately 60% of TEE represents the basal metabolic rate (BMR), which is mainly influenced by differences in LBM.55-57 In children BMR accounts for approximately 40 kcal/m²/hour. An additional 10% of TEE represents the thermogenic effect of food.58 The far most variable part of TEE is energy expenditure from physical activity.

Physical activity can be estimated by self-report methods such as questionnaires and activity diaries, and by objective techniques such as heart rate monitoring, motion sensors (accelerometer) and the doubly labelled water method. Self-reports are the most frequently used methods, but their validity in children has been questioned.59 Measurements with an accelerometer, which was used in the studies described in this thesis, have been found valid when tested against the doubly labelled water method in children60 and adolescents.61 Nevertheless, we need to keep in mind that accelerometer-registered activity is not the same as energy expenditure during physical activity.

Food intake is regulated by feeding and satiety centres in the hypothalamus, which are influenced by several different hormones. Leptin decreases energy intake, whereas cortisol and ghrelin, a circulating peptide hormone mainly produced by the stomach, stimulate food intake.62 Leptin is produced in adipose tissue, predominantly in subcutaneous fat,63, 64 and has been considered to be a signal to the hypothalamus when enough energy has been stored.62 Accordingly, leptin levels have been associated with FM and/or BMI in healthy adolescents65-68 and adolescents with type 1 diabetes.42, 67, 69-71

Leptin secretion is enhanced by insulin72, 73 and influenced by diet74 and sex steroids.75-77 Consequently, girls have been reported to have higher leptin levels, after adjustment for difference in body composition, compared to boys.57, 70 It has also been proposed that leptin may enhance energy expenditure, but this seems to be of less importance in children.66, 78

Energy intake can be assessed by observational methods such as 24-hour recall, food frequency questionnaires or prospective food diaries. It can also be estimated by quantitative methods, where the weights of different meals are measured. In the present studies we used a 7-day food diary that has been validated against weighed food records in adults.79
All methods are associated with several potential errors. Food frequency questionnaires and 24-hour recall are dependent on accurate memory of the consumed food, and subsequent underreporting of energy intake and selective nutrient intake is common. However, these methods interfere less with normal food habits than do prospective methods such as food diaries or weighed food records. The advantage of a food diary is that it allows direct assessment of food consumption and causes less interference with normal life compared to weighed food records.80

Type 1 diabetes during puberty

Adolescence is a time of rapid physical, emotional and psychological changes. During this period the child moves from complete dependence on the parents to a more independent lifestyle. For a teenager with diabetes this transition involves additional challenges. Responsibilities for insulin therapy, self-monitoring and meal planning have to move from the parents into the care of the teenager himself. In this section I will describe issues that are of specific importance for pubertal children with type 1 diabetes.

Glycaemic control

Glycaemic control often deteriorates during puberty, 38, 43, 81-85 especially in girls.4, 86-90 This deterioration occurs despite the use of increased dosages of insulin,4, 37, 38, 83, 87, 91 indicating decreased sensitivity to insulin during the pubertal years.

Insulin sensitivity

Insulin sensitivity decreases markedly during puberty in healthy adolescents, reaching its nadir in mid-puberty and returning to prepubertal levels in young adulthood.92, 93 This reduced insulin sensitivity is reflected by the increased plasma insulin levels that normally occur during puberty.94-96 A sex difference has frequently been described, with lower sensitivity to insulin in girls,93, 97 possibly reflecting differences in sex steroids and GH levels. The change in insulin sensitivity seems to be independent of changes in body fat.98

The reduction of insulin sensitivity is mainly mediated by increased GH secretion,97, 99 which acts by enhancing lipolysis and reducing glucose uptake in skeletal muscles. It has been suggested that the effect of GH on glucose uptake is mediated by an increase in free fatty acids (FFA) caused by enhanced lipolysis.100 During clamp studies it has been shown that the reduced insulin sensitivity affects the carbohydrate metabolism101, 102 mainly by decreasing peripheral glucose uptake, whereas the hepatic glucose
Insulin action on lipolysis and protein metabolism is not affected to the same extent during normal puberty.

Insulin sensitivity is even more impaired in adolescents with type 1 diabetes than in healthy adolescents and the impairment has been associated with glycaemic control. In accordance with findings in healthy adolescents, adolescent girls with diabetes are more insulin-resistant than boys. The resistance to insulin in adolescents with type 1 diabetes mainly affects the peripheral glucose uptake. However, the suppression of lipolysis mediated by insulin seems to be reduced in adolescents with poorly controlled type 1 diabetes. A disturbed GH/IGF-I axis has been suggested as a cause of this increased insulin resistance in adolescents with type 1 diabetes. Decreased insulin sensitivity has been associated with elevated GH levels and intervention with inhibited GH secretion has resulted in increased insulin sensitivity.

GH/IGF-I axis

Growth hormone secretion increases during puberty, reaching its maximum in Tanner stage III-IV in both sexes. GH exerts its effect not only directly but also by stimulating IGF-I production. Like GH, the IGF-I level increases during puberty, reaching its maximum during late puberty. IGF-I is mainly produced in the liver under the regulation of GH, insulin and nutritional factors. GH binds to the GH receptor, whereas insulin facilitates intracellular signalling and expression of the external part of the receptor (GH-binding protein).

Most of the produced IGF-I binds to specific binding proteins. IGF binding protein (IGFBP)-3 is the major binding protein, which together with the acid-labile subunit forms a complex that acts as an IGF-I depot. This complex can be cleaved by IGFBP-3 proteases to increase IGF-I bioavailability. However, the major regulator of IGF-I bioavailability is IGFBP-1. Although IGFBP-1/IGF-I complex only forms a small part of the total IGF-I, it is rapidly regulated because of the short half-life of IGFBP-1 and the regulatory effect of insulin on IGFBP-1 production.

IGF-I acts on its own receptors, which are small in number in adipose tissue, and on the insulin receptor. IGF-I seems to exert its effect on lipid metabolism by interacting with the insulin receptor. A recent study on adult patients with type 1 diabetes has shown that recombinant human IGF-I increases glucose uptake and decreases hepatic glucose production independently of insulin, although an effect on lipolysis was not demonstrated.

In adolescents with type 1 diabetes the GH/IGF-I axis is deranged, with low levels of IGF-I and IGFBP-3 and elevated levels of GH and IGFBP-1. GH-receptor resistance in the liver caused by low portal production remains normal. Insulin action on lipolysis and protein metabolism is not affected to the same extent during normal puberty.
insulin levels has been suggested as a possible mechanism behind this disturbance.\textsuperscript{33, 117} The consequence of these disturbances will be a decrease in IGF-I bioavailability, leading to enhanced GH secretion by a negative feed-back mechanism and deteriorated blood glucose regulation.\textsuperscript{118} Not surprisingly, these changes in the GH/IGF-I axis have been associated with poor glycaemic control.\textsuperscript{119, 120}

Disordered eating

Disordered eating behaviours are common in adolescent girls with type 1 diabetes, but not adolescent boys with this disease and have been associated with poor glycaemic control.\textsuperscript{121-127} an increased risk for microvascular complications,\textsuperscript{121, 126} high BMI\textsuperscript{40} and poor adherence to insulin therapy.\textsuperscript{123} The question of whether disordered eating behaviours are more common in adolescents with type 1 diabetes compared to controls has been extensively studied. The prevalence of disordered eating has been found to be increased among adolescents with type 1 diabetes,\textsuperscript{125} especially in diabetic girls.\textsuperscript{40, 123, 127} However, prevalence rates comparable to those in healthy controls have also been reported.\textsuperscript{3, 39} One possible reason why some studies have shown no difference between girls with diabetes and controls might be that the samples have been too small.\textsuperscript{128}

The most common weight control practice in diabetic adolescents, besides exercise and dieting, is reduction or omission of insulin injections.\textsuperscript{124} Previous studies have revealed that approximately 30 % of adolescent girls use less insulin than prescribed,\textsuperscript{39, 129} and 10-15% of adolescent girls have been reported to reduce their insulin dosage in an attempt to loose weight.\textsuperscript{121, 123, 124, 127} Omission of insulin has been associated with poor glycaemic control.\textsuperscript{129}

Ovarian function

Menstrual irregularities have been reported to be more common in girls with type 1 diabetes than in healthy control girls,\textsuperscript{130} and are associated with elevated HbA\textsubscript{1c},\textsuperscript{130, 132} and BMI.\textsuperscript{130} A higher frequency of polycystic ovarian syndrome among girls with type 1 diabetes has been suggested, on the basis of reports of decreased levels of sex hormone binding globulin (SHBG)\textsuperscript{132} and elevated testosterone levels\textsuperscript{133} in such girls.
Psycho-social factors

The family structure has frequently been claimed to be of importance for glycaemic control. Poor glycaemic control has been found to be associated with single-parent families\textsuperscript{134-136} and it is possible that this effect is due to the limited time available to supervise and support the child’s diabetes care.

There have been reports that girls with type 1 diabetes have lower self-esteem compared to boys with diabetes\textsuperscript{90} and to healthy girls,\textsuperscript{137} and that behaviour problems in adolescents predicts impaired glycaemic control during late adolescence and early adulthood.\textsuperscript{90}

Interventions

To my knowledge there have been no interventions aiming to prevent excessive weight gain in adolescents with type 1 diabetes. The purposes of the interventions reported have mainly been to improve glycaemic control and/or insulin sensitivity. Table 2 presents a summary of interventions in adolescents with type 1 diabetes, and is followed by a review of the use of the oral insulin sensitising agent metformin.

Table 2: Summary of interventions in adolescents with type 1 diabetes

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Glycaemic control</th>
<th>Insulin sensitivity</th>
<th>Weight</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhIGF-I\textsuperscript{138, 139}</td>
<td>+</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pirenzepine\textsuperscript{108, 140, 141}</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH antagonist\textsuperscript{142}</td>
<td>0</td>
<td></td>
<td></td>
<td>Reduced lipolysis and IGF-I</td>
</tr>
<tr>
<td>Intensified insulin therapy\textsuperscript{6}</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal insulin infusion\textsuperscript{143}</td>
<td></td>
<td></td>
<td></td>
<td>One 19 year old female. IGFBP-1 and GH amplitude decreased, IGF-I unchanged</td>
</tr>
<tr>
<td>Exercise\textsuperscript{144-151}</td>
<td>+/-0</td>
<td>+</td>
<td>-/0</td>
<td>Mostly increased weight. Increase in LBM?</td>
</tr>
<tr>
<td>Increased dietary monounsaturated fat\textsuperscript{152}</td>
<td>0</td>
<td></td>
<td></td>
<td>Poor dietary adherence</td>
</tr>
<tr>
<td>Coping skills training\textsuperscript{153}</td>
<td>+</td>
<td>+ (girls)</td>
<td></td>
<td>&lt; hypoglycaemia (girls)</td>
</tr>
</tbody>
</table>

+: beneficial effect; 0: no effect; -: negative effect.
Metformin
Different derivatives of guanidine were known during the nineteenth century, but it was not until 1922 that metformin (dimethylbiguanide) was first synthesised. During the years from 1960 to the early 1970s metformin was intensively studied, but was mostly used in France. In early 1970 phenformin (a derivative of guanidine similar to metformin) was found to be associated with severe cases of lactic acidosis. As a consequence the use of metformin was limited in the care of diabetic patients, and it was not until 1995 that this drug was introduced in the USA. It has since been found that in association with metformin, lactic acidosis occurs with a prevalence of 0.03 per 1000 patient years of treatment, mostly in elderly patients with renal insufficiency or cardiovascular failure.\textsuperscript{154}

After the positive effects of metformin in patients with type 2 diabetes had been presented from the UK Prospective Diabetes Study (UKPDS) in 1998, the clinical use of metformin increased dramatically.\textsuperscript{155} Today, metformin is regarded as first-line therapy in adolescents with type 2 diabetes and obese adults with type 2 diabetes.

Effects on glycaemic control and insulin dosage
Several studies have shown that metformin has a positive effect on glycaemic control in adults with type 2 diabetes.\textsuperscript{156} Recently, Jones et al.\textsuperscript{157} also demonstrated a beneficial effect on glycaemic control in adolescents with diabetes of this type. In studies on the effects of metformin in adult patients with type 1 diabetes, a reduced insulin requirement\textsuperscript{158-160} and a variable effect on glycaemic control have been found.\textsuperscript{159,161} The effect of metformin as additional therapy to insulin in children and adolescents with type 1 diabetes has been less frequently studied. The previous studies on metformin are summarised in Table 3.
Table 3: Studies of metformin in children and adolescents with type 1 diabetes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Protocol</th>
<th>Number</th>
<th>Age (years)</th>
<th>Glycaemic control</th>
<th>Dosage of insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fergusson 1961</td>
<td>XO DB</td>
<td>1+1</td>
<td>10</td>
<td>5.5-14</td>
<td>Reduced glucosuria</td>
</tr>
<tr>
<td>Schatz 1975</td>
<td>XO open</td>
<td>1+1</td>
<td>33</td>
<td>12.7(0.5)</td>
<td>No difference in blood glucose levels</td>
</tr>
<tr>
<td>Walravens 2000</td>
<td>RCT</td>
<td>6</td>
<td>80</td>
<td>15.5</td>
<td>Decreased HbA1c at 3 months but not at 6 months</td>
</tr>
<tr>
<td>Gomez 2002</td>
<td>UC</td>
<td>6</td>
<td>10</td>
<td>19.1(3.4)</td>
<td>No significant effect on HbA1c</td>
</tr>
</tbody>
</table>

XO = cross-over, DB = double-blind, UC = uncontrolled, RCT = randomised controlled study, ns = non-significant.

Effects on insulin sensitivity

Metformin has both direct effects and insulin-dependent effects on glucose metabolism. It has been suggested that the main mode of action is through a reduction of hepatic glucose production and an increase in peripheral glucose uptake. It has also been proposed that metformin enhances insulin effects on lipolysis and decreases fatty acid oxidation and intestinal glucose absorption, but the contributions of these effects to the total antihyperglycaemic action are considered to be small. Metformin reduces hepatic glucose production in patients with type 2 diabetes by reducing gluconeogenesis or glycogenolysis. Studies using the hyperinsulinaemic euglycaemic clamp technique have shown increased glucose uptake during metformin therapy compared to placebo in adults with type 1 diabetes, adolescents with PCO, and adult relatives of patients with type 2 diabetes. The effect of metformin on peripheral insulin sensitivity to glucose metabolism is mediated by increased translocation of glucose transporters (GLUT-1 and GLUT-4). The effect of metformin on fat metabolism seems unclear, although there have been reports of reduced lipolysis and decreased fat oxidation during metformin treatment.
Aims

The overall aim of the investigations summarised in this thesis was to increase the understanding of the excessive weight gain observed in adolescents with type 1 diabetes and to examine the effect of metformin as additional treatment to insulin in adolescents with poor glycaemic control.

The specific aims were:

- To describe the growth of children and adolescents with type 1 diabetes and to compare the body mass index at late adolescence in type 1 diabetes with that of healthy controls (Study I).

- To compare the total body fat content and fat distribution in adolescent girls with type 1 diabetes with those in healthy age-matched girls (Study II).

- To compare the physical activity level and energy intake in adolescent females with type 1 diabetes in comparison with healthy age-matched controls (Study III).

- To investigate the relationships of physical activity and energy intake to change in body composition in adolescent girls with type 1 diabetes (Study IV).

- To study the effect of metformin as additional treatment to insulin on glycaemic control and insulin sensitivity in adolescents with poorly controlled type 1 diabetes (Study V).
Research design and methods

Research design

Study I (Paper I)
A prospective case-control study was performed between the ages of 18 and 22 years, where data for height, weight, daily dosages of insulin and HbA1c were collected in all subjects who were transferred from the Department of Paediatrics to the Department of Internal Medicine during the years 1990–94. Additionally, height, weight, pubertal development, daily dosages of insulin, and HbA1c were recorded retrospectively from the diagnosis of diabetes to the age of 18 years. Only data in subjects with a prepubertal diagnosis were considered when the associations between pubertal glycaemic control and insulin dose and growth were examined. The healthy controls were matched for sex and age and recruited from a comprehensive school in Örebro.

Study II (Paper II)
Body composition was measured by dual-energy X-ray absorptiometry (DXA) and compared between adolescent girls with prepubertal onset of type 1 diabetes and healthy age-matched girls in a cross-sectional case-control study. All subjects with diabetes were recruited from the outpatient clinic of the Department of Paediatrics in Örebro. The control subjects were identified as the female of the same age appearing next to the female with diabetes in the register of the county taxation authorities.

Study III (Paper III)
A cross-sectional case-control study was conducted for comparison of food habits and physical activity between adolescent girls with type 1 diabetes and healthy age-matched girls. Food habits were measured with a 7-day food diary, and physical activity was measured for 7 days with an accelerometer. All girls aged 12–19 years at the Department of Paediatrics in Örebro who had had type 1 diabetes for more than 2 years were asked to participate in the study. Control girls were identified from the register of the county taxation authorities and were matched for age and residential area.
**Study IV (Paper IV)**

The associations between food habits (7-day food diary) and physical activity (accelerometer) and changes in body composition (DXA) in adolescent girls with type 1 diabetes and healthy age-matched girls were investigated in a longitudinal case-control study. For this purpose 23 girls with type 1 diabetes and 19 healthy age-matched controls from study III were followed up for one year.

**Study V (Paper V)**

A randomised double-blind placebo-controlled study was performed to investigate the effects of 3 months of adjunctive therapy with metformin in adolescents with poorly controlled type 1 diabetes. The primary outcome measure was HbA1c, which was assessed on inclusion in the study and thereafter every month during the study period. The secondary outcome variables, namely insulin sensitivity, IGF-I, IGFBP-1, daily insulin dosage, BMI, waist circumference, and blood lipid concentrations, were measured on inclusion and at the end of the study period.

Participants were recruited from five different Departments of Paediatrics in central Sweden (Eskilstuna, Falun, Karlstad, Västerås and Örebro), during the period from November 1998 to March 2001. Girls aged 14–20 years and boys aged 16–20 years, with an HbA1c level above 8% and a daily dosage of insulin > 0.9 U/kg, were asked if they would participate in the study. A total of 30 adolescents were recruited, according to the defined criteria. There were 12 from Örebro, and 5, 4, 5 and 4 from Eskilstuna, Falun, Karlstad and Västerås, respectively.

The included subjects were randomised to receive metformin or placebo for 3 months. The initial study dose was 500 mg daily in the morning for one week, and this was followed by 500 mg twice daily for 3 weeks, and subsequently 1000 mg twice daily for the rest of the study period.

**Subjects**

The characteristics of the included subjects are described in Table 4. Most of the participants were recruited from the Department of Paediatrics at the University Hospital of Örebro, where outpatient and hospital care for all children and adolescents with diabetes in the county of Örebro is provided. The county of Örebro has a population of 274 000 inhabitants (65 000 below the age of 19) and at the end of the year 2003 the number of children and adolescent with diabetes receiving care at the paediatric diabetic clinic was 220. All patients included in the studies had type 1 diabetes mellitus and had a clinical history of typical diabetes symptoms at onset, and all of them required insulin treatment from the onset of diabetes.
Table 4: Clinical characteristics of the included subjects

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Sex</th>
<th>Number</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Daily dosage of insulin (U/kg)</th>
<th>HbA₁c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Type 1 diabetes</td>
<td>Girls</td>
<td>45</td>
<td>18.2 (0.2)</td>
<td>23.9 (2.7)</td>
<td>0.9 (0.2)</td>
<td>8.8 (2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boys</td>
<td>44</td>
<td>22.4 (2.4)</td>
<td>1.0 (0.2)</td>
<td>8.6 (1.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Girls</td>
<td>45</td>
<td>18.0</td>
<td>21.2 (1.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boys</td>
<td>44</td>
<td>22.2 (2.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Type 1 diabetes</td>
<td>Girls</td>
<td>18</td>
<td>17.3 (0.6)</td>
<td>26.3 (2.6)</td>
<td>1.1 (0.3)</td>
<td>8.0 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>Girls</td>
<td>18</td>
<td>17.3 (0.6)</td>
<td>23.6 (3.8)</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Type 1 diabetes</td>
<td>Girls</td>
<td>26</td>
<td>15.7 (2.1)</td>
<td>23.3 (3.3)</td>
<td>1.1 (0.3)</td>
<td>7.6 (1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>Girls</td>
<td>49</td>
<td>15.8 (2.1)</td>
<td>22.0 (3.8)</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>Type 1 diabetes</td>
<td>Girls</td>
<td>23</td>
<td>15.7 (2.1)</td>
<td>23.0 (3.1)</td>
<td>1.1 (0.3)</td>
<td>7.6 (1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td>Girls</td>
<td>19</td>
<td>15.6 (1.9)</td>
<td>22.7 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>Type 1 diabetes</td>
<td>Girls</td>
<td>18</td>
<td>16.8 (1.7)</td>
<td>25.7 (3.7)</td>
<td>1.2 (0.2)</td>
<td>9.7 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boys</td>
<td>8</td>
<td>17.4 (1.3)</td>
<td>21.4 (3.2)</td>
<td>1.3 (0.3)</td>
<td>9.2 (1.2)</td>
</tr>
</tbody>
</table>

Values are mean and SD.

Withdrawals

Study I

Growth charts from diagnosis to the age of 18 were available in 74 patients (85 %), whereas 50 (26 boys and 24 girls) of these had a prepubertal onset of diabetes. Prospective longitudinal data were obtained from 65 diabetic patients (73 %) between the ages of 18 and 22. Sixteen had moved from the county and 8 had not reached the age of 22 at the time of analysis. Of the control adolescents 46 (67%) were re-evaluated at the age of 22.

Study II

Two females with diabetes were asked to participate but declined for practical or geographical reasons.

Six of the first 18 controls refused and each was therefore replaced by the next person in the register who was willing to participate, in practice the second, third or fourth control person. There were no significant differences in height, weight or BMI between the group of first control subjects and the six subjects who replaced those who refused to participate.

Study III

Four girls were excluded because of a severe concurrent disease (mental retardation or rheumatoid arthritis). Of the eligible girls, 26 (67 %) agreed to participate. The subjects who refused to participate did not differ from the study population in age, BMI, insulin dosage or HbA₁c.
Study IV
Three girls with type 1 diabetes could not be followed up, one because she had moved from the catchment area. The subjects who were lost to follow-up had a mean age of 15.5 years, mean BMI of 25.4 kg/m², mean HbA₁c of 8.5 % and a mean daily insulin dosage of 1.0 U/kg.

Twenty-three controls were recruited, matched for age and residential area. Nineteen of the control girls agreed to participate in the follow-up. Those who were lost to follow-up had a mean age of 17.6 years and a mean BMI of 23.9 kg/m².

Study V
In the Department of Paediatrics in Örebro, a total of 51 eligible adolescents were identified, and 20 of them (39%) fulfilled the inclusion criteria. Twelve agreed to be included in the study. The non-participating adolescents did not differ from the participants regarding age, daily dosage of insulin, BMI or HbA₁c. In the other centres, which had a comparable number of patients, and comparable treatment guidelines and mean HbA₁c, the participation rates were lower because of the need to travel by car or train to the clinic in Örebro.

Methods

Blood analyses

Blood Glucose
Glucose dehydrogenase technique
(HemoCue AB, Ångelholm, Sweden)

S-Cholesterol
Photometric method (Vitros 950 instrument, Johnson & Johnson Clinical Diagnostics, Rochester, USA). Intra-assay CV 1.5% at 7.0 mmol/l.

HbA₁c
High performance liquid chromatography using the Mono S standard with reference range 3.5-5.3%. The intra- and interassay CV were 1.3 and 1.1%, respectively. Affinity chromatography was used before 1991 and these values were considered to be linearly related to those obtained by HPLC and therefore converted.

S-Insulin
Fluoroimmunometric assay (Wallac Oy Insulin kit for AutoDELFIATM, Turku, Finland). Intra-assay CV was 1.8% at 50 µU/ml and 2.9% at 125 µU/ml and inter-assay CV was 3.5%.
S-Insulin-like growth factor-I  IRMA technique (Nichols Institute Diagnostics, San Clemente, California, USA). Intra-assay CV 6.7 % at 207 µg/l.

S-IGF binding factor-1  IRMA technique (Diagnostic Systems Laboratories, Inc., Webster, California, USA). Intra-assay CV was 8.9% at 52 µg/l.

S-HDL  Photometric method (Hitachi 911 instrument, Roche Diagnostics, Basel, Switzerland). Intra-assay CV was 1.7% at 1.4 mmol/l.

S-Leptin  RIA (Linco Research, Inc., Missouri, USA) Intra-assay CV was 5.6% at 20.5 µg/l.

S-LDL  Photometric method (Hitachi 911 instrument, Roche Diagnostics, Basel, Switzerland). Intra-assay CV was 2.6% at 3.3 mmol/l.

S-Sex hormone binding globulin  Fluoroimmunometric method (Wallac OY, Turku, Finland). Intra-assay CV was 0.9-1.3% at a level of 28-173 nmol/l.

S-Testosterone  RIA (Diagnostics Product Corporation, Los Angeles, USA). Intra-assay CV was 7.0% at 3.8 nmol/l.

S-Triglyceride  Photometric method (Vitros 950 instrument, Johnson & Johnson Clinical Diagnostics, Rochester, USA). Intra-assay CV was 3.6% at 2.5 mmol/l.

Hyperinsulinaemic euglycaemic clamp
The patients arrived at the Department of Paediatrics of the University Hospital, Örebro in late afternoon. Two indwelling catheters were inserted, one in the antecubital vein for intravenous insulin infusion and the other in a dorsal hand vein for blood sampling. The patients fasted from 20.00 hours that evening. During the night the patients received a variable insulin infusion, and their blood glucose was measured each hour, with the aim of achieving normoglycaemia during the night. At 07.00 – 08.00 hours in the morning, fasting blood samples were drawn and the hyperinsulinaemic euglycaemic clamp procedure was initiated.

A primed infusion of human soluble insulin (Actrapid® , Novo Nordisk, Copenhagen, Denmark) was given at a rate of 40 mU.m⁻².min⁻¹ for at least 60 minutes to achieve steady state. During the insulin infusion, the blood glucose was kept constant at 5.0 mmol/l by a variable infusion of 20% glucose. The rate of glucose infusion was adjusted manually according to
the blood glucose levels, which were measured every 5 minutes. If the blood glucose was unstable after 60 minutes, the insulin infusion was prolonged for 15–30 minutes until steady state was achieved. Blood samples for serum insulin measurements were taken at the beginning of steady state and then after 20, 40 and 60 minutes. Samples for blood glucose and insulin were taken from a heated superficial hand vein, to obtain arterialised samples.

The amount of glucose infused during the last 60 minutes after achievement of steady state was calculated (M value = mg.m⁻².min⁻¹) and was related to the mean insulin concentration during steady state for estimation of insulin sensitivity (M/I value = mg.m⁻².min⁻¹ x µU.ml⁻¹).

**Body composition measurements**

*Anthropometry*

Height and weight were measured in all studies and BMI was calculated as weight/height². Target height was calculated from sex-adjusted mean parental heights, ± 13 cm, in study I. Standard deviation scores (SDS) for weight and height were calculated in study I using national Swedish growth charts published in 1976.¹⁷⁷ BMI was compared with Swedish nationwide reference graphs published in 1995¹⁷⁸ in study I and with BMI reference curves based on measurements on British children¹⁷⁹, also published in 1995, in studies III and IV.

Waist and hip circumferences were measured, the waist at the umbilicus and the hip at its widest point.

*Skinfold measurements*

Skinfold thickness was measured with a Harpenden’s caliper (British Indicators Ltd, West Sussex, UK) at the biceps and triceps and at subscapular and supra-iliac areas as described by Lohman et al.¹⁸⁰ Three sets of measurements were made at each site and the mean values were used. All the measurements were made by the same investigator. Percentage body fat was calculated from triceps and subscapular skinfolds using the equation of Slaughter et al.¹⁸¹

*Dual-energy X-ray absorptiometry*

Total body composition was measured by DXA with a Lunar DPX-L scanner (Lunar Corp, Wisconsin, USA) that uses a pencil-beam technique. The adult scan mode was used in all studies. All DXA measurements were performed in the fasting state in the morning before breakfast to minimise differences in hydration.

The coefficients of variation for fat measurements as determined by three measurements on each of three phantoms with a fat content of 10, 20 and 40 kg were 10.4%, 1.7% and 0.3 %, respectively.

Abdominal fat measurement was performed by choosing a region of
interest. This was restricted by the inner margins of the lower ribs vertically and by the most cranial point of the second lumbar vertebra (L2) and the most caudal point of the fourth lumbar vertebra (L4) horizontally. The fat distribution was described by the ratio between abdominal fat and leg fat. Lean body mass was calculated as body weight minus fat mass and bone mineral content. The standard deviation scores for percentage body fat and lean body mass were calculated as described by van der Sluis et al.46

**Pubertal stage**

Pubertal development was assessed from Tanner stages in studies III and IV. We used a self-estimating manual (“Flickboken”), using schematic drawings of different Tanner stages, which have been found to show good accordance with professional estimations. Similar self-assessment methods have been reported to be valid for use in adolescents.184, 185

**Total physical activity**

Physical activity was assessed with a uniaxial accelerometer (model WAM 6471; Computer Science and Applications Inc, Shalimar, FL). The subjects were asked to wear the accelerometer during the daytime, except during water activities, during a 7-day period. The accelerometer was secured directly to the skin, with an elastic belt, on the lower part of the back (L 4–5) or at the hip. Activity data were analysed and processed with the use of a specially written macro based on Microsoft ACCESS (Microsoft Inc, Redmond, WA).

Data were described as total physical activity, accumulated time spent sedentarily, and accumulated activity at low intensity and at moderate or higher intensity. The cut-off points for low (100-1952 counts/min), moderate, and higher intensity (>1952 counts/min) were adopted from Freedson et al.186 To take into account inter-individual differences in registration time, the total physical activity was expressed as total counts divided by registered time, i.e. counts · min⁻¹ · day⁻¹. All activity data were averaged over the 7-day period. The individual registrations were included in the analysis if 5 or more days had more than 600 minutes of recording. Only days with more than 600 minutes were included in the analysis of total physical activity. In the analysis of physical activity hour-by-hour, every hour with less than 30 minutes of registration were excluded.

**Food intake**

A 7-day food diary (“Menyboken”, Swedish National Food Administration) was used to estimate food intake. The diary is constructed as a booklet with printed lists of foods for breakfast, lunch, dinner and snacks and pictures of different sizes of meals. A space was left to record foods not present on the list. The subjects were asked to fill in the diary directly after the meal, except for the lunch meal, which was recorded in the afternoon.
after school. The subjects with diabetes were also asked to record the food consumed during hypoglycaemic episodes on a separate list. The diaries were subsequently analysed using designed software (“Dietist”, Kost och Näringsdata AB, Bromma, Sweden).

Nutrient intakes were calculated as means of the 7 study days. Energy intakes derived from carbohydrates, fat and protein were adjusted for total energy intake (E%).

Statistical methods

All calculations were performed with StatView version 5.0.1. The data distribution was examined for all variables using histograms and descriptive statistics. The level of statistical significance was set at P< 0.05.

Study I
An unpaired t-test was used for comparison between continuous variables and a $\chi^2$ test for comparison of frequency distributions. A paired t-test was used to evaluate differences between two measurements in the same group. Associations between variables were tested for significance with Pearson’s product moment correlation coefficient and Fisher’s r to z.

Study II
A paired t-test was used to assess the significance of differences between girls with diabetes and controls. The associations between variables were tested for significance with Pearson’s product moment correlation coefficient and Fisher’s r to z.

A Bland-Altman plot was constructed for comparison between body fat values calculated from skinfold measurements and those obtained by DXA. In this graph the mean of the two measurements is displayed against the difference between the two measurements. The mean difference and the limits of agreement (mean ± 2SD for the difference) were calculated. The possibility of a relation between the difference and the mean of the two methods was tested by linear regression.

Study III
Analysis of covariance (ANCOVA), with study group (diabetic versus control) as fixed factor and age as covariate, was used to examine differences in physical activity variables, energy intake and clinical characteristics between the groups. When the data distribution was skewed, we used median and interquartile range (IQR) to describe the data and the Mann-Whitney U test to compare the groups. Linear regression analyses were performed to assess the relations of physical activity and energy intake to metabolic control and body composition.
Study IV

An unpaired t-test or the Mann-Whitney U test was used to compare the groups regarding data on clinical characteristics, body composition, physical activity and energy intake. Analysis of covariance, with study group as fixed factor, was used to analyse the associations between change in body composition variables (fat mass, percentage fat mass and lean body mass) and energy intake, physical activity and leptin levels. Leptin levels and extra energy intake due to hypoglycaemia were not normally distributed and were therefore log transformed before being analysed. In all analyses adjustments were made for Tanner group and body composition (fat mass or LBM) at baseline. Interactions between study group, Tanner group and energy-adjusted fat intake or total physical activity were examined. The results of the ANCOVA were presented as regression slope (\( \beta \)-coefficient).

The association, in the diabetic group, between daily dosage of insulin, HbA1c, and extra energy intake due to hypoglycaemia and change in body composition variables was analysed by multiple regression analysis with change in body composition as dependent variable and adjustment for body composition at baseline.

Study V

We estimated that with an SD of 1.0% for HbA1c, a two-sided 0.05 significance level and a power of 80%, overall sample sizes of 18 and 34 subjects would be sufficient to detect true treatment differences of 1.5% and 1.0%, respectively.

A paired Student’s t-test or Wilcoxon signed-rank sum test was used to estimate the difference between baseline and the end of the study. An unpaired t-test or the Mann-Whitney U test was used to test differences between the two groups. A 95% confidence interval (CI) and/or the P value was calculated when appropriate.

Pearson’s product moment correlation coefficient and Fisher’s r to z was calculated to estimate associations between change in HbA1c, change in insulin sensitivity and different variables.
Results

Longitudinal assessment of growth (Paper I)

**Height**
The height SDS at diagnosis of diabetes (+0.17 ± 1.10) exceeded the final height SDS (P < 0.05). Maximal growth velocity occurred at the age of 11 in the diabetic girls (6.6 ± 1.4 cm/year) and at 13 in the diabetic boys (7.3 ± 2.1 cm/year). Height SDS decreased by 0.39 SDS in the diabetic boys and by 0.34 SDS in the diabetic girls between the ages of 15 and 18 (P < 0.01). Height at the age of 18 showed no difference between diabetic boys (177.7 ± 7.4 cm) or girls (165.1 ± 6.2 cm) and healthy boys and girls of the same age.

**Weight and BMI**
There was a marked sex difference, with an increase in weight SDS and BMI in the diabetic girls during the years following menarche. At the age of 18, the BMI of the diabetic girls (23.9 ± 2.7 kg/m²) was higher than that of the control girls (21.2 ± 1.8 kg/m²) (P < 0.001), and the mean weight of the diabetic girls was 6.5 kg higher than that of the control girls (P < 0.001). Among the boys, there was no difference in BMI between diabetic patients and controls at the age of 18 and the weight gain between the ages of 18 and 22 was comparable in the two groups.

After the age of 18, the weight of the diabetic girls was relatively constant. The increase in weight by 1.6 kg between the ages of 18 and 22 was not significant. In the control girls, on the other hand, the body weight increased significantly by 3.2 kg between these ages (P < 0.01).

**Longitudinal assessment of glycaemic control and dose of insulin**
The daily dosage of insulin was higher in girls than in boys during the age-period 11–13 years, and reached a maximum of approximately 1.0 U/kg/day in both sexes. During late adolescence the insulin dose was higher in boys.

There was no significant sex difference in HbA₁c during puberty. In the girls HbA₁c improved significantly, by 1.6 % from the age of 18 to the age of 22 (P < 0.001), whereas in the boys there was no significant improvement.
Associations between glycaemic control, dosages of insulin and growth

Mean HbA1c during puberty was negatively related to final height SDS \( (r = -0.40; P < 0.05) \) and positively related to difference between final height and target height \( (r = 0.31; P < 0.05) \) in girls but not in boys. However, glycaemic control during puberty was not significantly correlated with the difference between height SDS at diagnosis and final height SDS in either boys or girls. No correlation was found between duration of diabetes and final height SDS.

Mean HbA1c during puberty \( (r = 0.47; P < 0.01) \), but not mean dosage of insulin, was positively related to BMI at the age of 18 in girls. In boys we found no associations between average glycaemic control or dosage of insulin during puberty and BMI at the age of 18.

Total body fat mass and fat distribution (Paper II)

Total body fat mass

The total body fat mass was measured by DXA in studies II and IV. In study II total body fat mass was 5.3 kg higher \( (P < 0.05) \) in the females with type 1 diabetes than in the controls and the percentage body fat was 37.1 ± 5.5 % in the diabetic patients and 32.1 ± 7.7 % in the controls \( (P < 0.01) \)(Table 5). There was no difference in lean body mass between the groups. In study IV, where the girls were younger, there was a tendency towards a higher fat mass in girls with diabetes, but with no significant difference between the groups.

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Age (years)</th>
<th>Percentage body fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1 diabetes</td>
<td>17.3 (0.6)</td>
<td>37.1 (5.5)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>17.3 (0.6)</td>
<td>32.1 (7.7)</td>
</tr>
<tr>
<td>IV</td>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 1 diabetes</td>
<td>15.7 (2.1)</td>
<td>32.6 (8.3)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>15.6 (1.9)</td>
<td>31.0 (9.9)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 1 diabetes</td>
<td>16.8 (2.1)</td>
<td>35.0 (6.5)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>16.6 (1.9)</td>
<td>32.7 (8.4)</td>
</tr>
</tbody>
</table>

Values are mean and SD.

Fat distribution

The amount of arm and torso fat were significantly greater in the girls with type 1 diabetes than in the controls \( (P < 0.05) \), whereas the amounts of abdominal and leg fat did not differ significantly between these two groups. Neither the ratio of abdominal fat to total fat nor the ratio of abdominal fat
to leg fat showed any significant differences between these groups. There was no difference between these groups regarding waist or hip circumference or the ratio between these two measurements.

**Hormones**

There was a tendency towards a higher testosterone level and a higher testosterone/SHBG ratio in the diabetic girls compared to the controls, but these differences did not reach statistical significance (Table 6). There was no correlation between abdominal-to-leg ratio and testosterone/SHBG ratio or testosterone level.

Table 6: Serum hormone and lipid concentrations in adolescent girls

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetes</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.7 (1.2)</td>
<td>1.0 (0.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>72.1 (59.6)</td>
<td>84.8 (65.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Testosterone/SHBG</td>
<td>3.9 (4.0)</td>
<td>2.1 (1.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.8 (1.1)</td>
<td>4.3 (0.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.5 (0.3)</td>
<td>1.4 (0.3)</td>
<td>ns</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.6 (1.0)</td>
<td>2.3 (0.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.4 (1.0)</td>
<td>1.1 (0.5)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are presented as mean and SD. SHBG = sex hormone binding globulin.

**Associations between body composition and metabolic variables**

There were significant positive associations between indices of abdominal fat and glycaemic control and lipid levels in girls with type 1 diabetes, but overall body fat (BMI, %BF) did not correlate with HbA1c or lipid levels (Table 7).

Table 7: Pearson correlation coefficients between body composition and metabolic variables in adolescent girls with type 1 diabetes

<table>
<thead>
<tr>
<th></th>
<th>HbA1c (%)</th>
<th>Daily dosage of insulin (U/kg)</th>
<th>Total cholesterol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.25</td>
<td>0.005</td>
<td>0.30</td>
<td>0.28</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.51*</td>
<td>0.29</td>
<td>0.49*</td>
<td>0.46</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.60**</td>
<td>0.37</td>
<td>0.71***</td>
<td>0.62**</td>
</tr>
<tr>
<td>Abdominal-to-leg ratio</td>
<td>0.69***</td>
<td>0.78****</td>
<td>0.60**</td>
<td>0.73****</td>
</tr>
<tr>
<td>Abdominal fat (kg)</td>
<td>0.58*</td>
<td>0.39</td>
<td>0.57*</td>
<td>0.51*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.62**</td>
<td>0.62**</td>
<td>0.50*</td>
<td>0.77****</td>
</tr>
<tr>
<td>Daily dosage of insulin (U/kg)</td>
<td>0.62**</td>
<td>-</td>
<td>0.38</td>
<td>0.62**</td>
</tr>
</tbody>
</table>

* P<0.05; **P<0.01; ***P<0.005; ****P<0.001; *****P<0.0005.
Comparison between fat mass estimated from skinfold measurements and that measured by DXA

Percentage body fat estimated from skinfold measurements was significantly higher in girls with diabetes than in controls (40.2 ± 8.9 % and 29.1 ± 8.1 %, respectively; P < 0.01). There was a strong correlation between %BF from skinfold measurements and that obtained by DXA (controls: r = 0.88, P < 0.0001; diabetic females: r = 0.90, P < 0.0001). However, using a Bland-Altman plot it was demonstrated that skinfold measurements overestimated the amount of body fat in diabetic patients, especially in those who had a high fat mass (Fig. 1).

Figure 1 Bland-Altman plot showing the association between the difference in estimated percentage body fat (skinfold - DXA), and the mean percentage body fat in girls with type 1 diabetes (A) and control girls (B). Mean values ± 2 SD for the difference are given in the figure. There was a positive correlation between the difference and the mean percentage body fat (r = 0.76; P < 0.0001) in girls with diabetes. The association is also described by the regression line (difference in percentage body fat = -16.1 + 0.49 mean %BF; R² = 0.58).
Total physical activity and energy intake (Paper III)

**Physical activity**

The total amount of physical activity seemed to be lower in the diabetic group (464 ± 123 counts · min⁻¹ · day⁻¹) than in the control group (523 ± 138 counts · min⁻¹ · day⁻¹), but the difference between the study groups did not reach statistical significance (P = 0.06). The girls with diabetes recorded more time spent sedentarily compared to the controls (443 ± 60 and 390 ± 73 min/day, respectively)(P < 0.01). Age influenced all variables of physical activity and total physical activity was inversely related to age in both diabetic girls and controls.

Physical activity at different time-points during awake hours on weekdays is presented in Figure 2. There were no significant differences in physical activity between the groups either during school time, after school or during the weekends.

*Figure 2* Physical activity during awake hours in adolescent girls with type 1 diabetes and healthy control girls.
**Energy intake**

The total daily energy intake, with or without adjustment for body weight, showed no difference between the groups. The nutrient intake in girls with diabetes and the control girls are presented in Table 8.

Energy intake in per cent of predicted TEE was 84% in the diabetic groups and 86% in the control group, indicating no difference between the groups in their reliability of reporting energy intake. However, there was a significant effect of age, with higher energy intake in per cent of predicted TEE in girls less than 16 years of age, without interaction with study group. BMI SDS was inversely associated with total energy intake expressed as a percentage of predicted TEE in the control girls (reported energy intake in per cent of predicted TEE (%) = 93 – 15.1BMI SDS; P = 0.0001) but not in the diabetic girls (reported energy intake in per cent of predicted TEE (%) = 85 - 1.4BMI SDS; P = 0.75), indicating that overweight control girls seem to underreport their energy intake more than overweight girls with diabetes.

Use of non-light soft drinks was reported by 54% of the diabetic girls and 98% of the controls. The average daily intake of energy from non-light soft drinks was significantly higher in the control group (type 1 diabetes: median 49 kJ/d, IQR 245 kJ/d; controls: median 380 kJ/d, IQR 683 kJ/d; P < 0.0001). There was no significant correlation between consumption of soft drinks and age in either of the groups.

Fourteen girls with diabetes reported intake of extra meals because of hypoglycaemia, ranging from 1 to 5 extra meals per week. The median extra energy intake was 3.3 MJ/week, ranging from 1.2 to 6.4 MJ/week. As expected, there was a significant correlation between extra energy intake and total energy intake per day (r = 0.55; P < 0.01), which means that those with frequent hypoglycaemia had a higher total energy intake. There was no significant age difference between girls who reported extra meals and those who did not.

**Table 8: Food intake in girls with type 1 diabetes and healthy control girls**

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetes</th>
<th>Controls</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total energy intake</strong> (MJ/d)</td>
<td>8.5 (1.8)</td>
<td>8.4 (2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (E%)</td>
<td>34 (6)</td>
<td>33 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (E%)</td>
<td>50 (6)</td>
<td>53 (4)</td>
<td>Study group*</td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>16 (2)</td>
<td>14 (3)</td>
<td>Study group**</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>20 (7)</td>
<td>16 (6)</td>
<td>Study group*</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). * = P < 0.05, ** = P < 0.01, *** = P < 0.001. NS = non-significant.
Associations with glycaemic control and insulin therapy

There were no associations between physical activity and daily dosage of insulin or glycaemic control.

Daily dosage of insulin was not related to total energy intake in girls with diabetes. There were no difference in total energy intake between girls treated with 4 and those treated with 6 insulin doses per day. Total energy intake was not associated with glycaemic control.

Relationships between physical activity, food intake and change in body composition (Paper IV)

Change in percentage body fat and change in body fat mass were positively associated with energy-adjusted fat intake ($P < 0.01; P < 0.05$, respectively) (Fig. 3) and inversely associated with energy-adjusted carbohydrate intake ($P < 0.01, P < 0.01$, respectively), without any interaction between study group and energy-adjusted fat intake. Total physical activity (counts · min$^{-1}$ · day$^{-1}$), total energy intake or extra energy intake due to hypoglycaemias were not associated with change in percentage body fat or change in body fat mass.

Change in lean body mass was not associated with energy-adjusted carbohydrate or fat intake, but was positively associated with the total amount of physical activity ($\beta = 0.006$ (kg/counts · min$^{-1}$ · day$^{-1}$), ($P < 0.01$), without any observed interactions with study group.
Figure 3 The association between energy-adjusted fat intake and annual change in percentage body fat in adolescent girls with type 1 diabetes and healthy control girls. Change in % body fat = -9 + 0.33 E% fat; $R^2 = 0.33, P < 0.0001$.

**Associations between insulin therapy, HbA1c and change in body composition**

There were no significant associations between daily dosage of insulin or HbA1c and change in percentage body fat or change in lean body mass. Using the unpaired t-test a significant difference was found between the annual increase in percentage body fat in those treated with 4 daily dosages of insulin ($N = 9$) compared to those with 6 doses ($N = 6$) ($0.8 \pm 2.6\%$ units and $3.6 \pm 2.1\%$ units body fat, respectively, $P < 0.05$).

**Associations between leptin levels and body composition**

There was no significant difference in leptin levels between the groups with and without adjustment for percentage body fat. Log transformed leptin levels were positively associated with percentage body fat at baseline (diabetic girls: $P < 0.0001$; controls: $P < 0.0001$) and at follow-up (diabetic girls: $P < 0.0001$; controls: $P < 0.0001$). Baseline leptin and mean leptin levels during the study were not associated with change in percentage body fat.
Effects of additional therapy with metformin (Paper V)

**Glycaemic control**
During the study period the mean HbA1c value decreased from 9.6 ± 1.0 to 8.7 ± 1.5 % (CI for the change: -1.6 to -0.1; P < 0.05) in the metformin group, but remained unchanged (9.5 ± 1.2 vs. 9.2 ± 1.3; ns) in the placebo group. There was no significant difference between the groups in change in HbA1c (P = 0.17). In neither of the groups did the daily insulin dosage change significantly during the 3-month trial. There were no significant gender differences in improvement in HbA1c or change in insulin dose.

In the metformin group, change in HbA1c showed no association with the initial values for HbA1c, insulin dosage or change in insulin sensitivity. However, there was an inverse correlation between M/I on inclusion and change in HbA1c (r = -0.62; P < 0.05), indicating that patients with decreased initial insulin sensitivity benefited most from metformin.

**Insulin sensitivity**
The M values were unchanged in both groups. M/I, however, increased significantly in the metformin group during the study (P < 0.05), but was unchanged in the placebo group. In the metformin group, change in insulin sensitivity (M/I) showed no association with initial HbA1c, insulin dosage or change in insulin dose. However, there was a significant positive association between change in insulin sensitivity and initial M/I (r = 0.77; P < 0.01), indicating again that patients with lower initial insulin sensitivity benefited most from metformin treatment.

**Additional findings**
There were no significant changes in BMI, waist circumference, blood lipid levels or IGF-I in either the metformin or the placebo group. IGFBP-1 seemed to decrease in the metformin group, but this change did not reach statistical significance. Gastrointestinal side effects were not more common in the metformin group.

Insulin sensitivity at baseline was not associated with BMI SDS. However, the waist-hip ratio was inversely related to M/I (r = -0.58; P < 0.01).
Discussion

Hypothesis for weight gain and accumulation of body fat

In study I we found an excessive weight gain in girls, but not in boys, with type 1 diabetes compared to controls, and that the increase in BMI started around the menarche. This is in accordance with most previous reports.4, 24, 32, 35-40 We observed, further, that the weight difference in girls was diminished between the ages of 18–22 years. The underlying reasons for this excessive weight gain are not fully understood although several potential mechanisms have been suggested, namely: intensification of insulin therapy, increased energy intake due to hypoglycaemia, decreased physical activity or leptin resistance.

Currently there is a trend towards overweight among children and adolescents.188-190 Whether this is also true in adolescents with type 1 diabetes has been less well investigated, although there is some evidence that BMI is increasing191 even in this population.

Insulin therapy

Excessive weight gain has been associated with daily dosage of insulin,42, 91, 192 number of insulin injections per day24, 41, 91 and intensification of insulin therapy.6, 191

Weight gain associated with intensification of insulin therapy is accompanied by improved glycaemic control6, 193, 194 and it has been suggested that the cause of this weight gain is decreased glucosuria. In a prospective study on adults with type 1 diabetes, Carlson et al.195 found that elimination of glucosuria during intensive insulin therapy accounted for 70 % of an observed weight gain. On the other hand, there have been longitudinal studies that have failed to show an association between improved glycaemic control and weight gain during MIT,28, 41 implying that factors other than reduced loss of energy cause weight gain during MIT. This is supported by our finding in study I that the mean HbA1c level during puberty was positively associated with BMI at the age of 18 in girls with type 1 diabetes.
It has also been hypothesised that peripheral hyperinsulinaemia caused by unphysiological insulin therapy mediates the gain in weight. If this hypothesis is true, it could contribute to the understanding about the sex difference in body composition, since insulin requirements during puberty are higher in girls than in boys.\textsuperscript{87} Hyperinsulinaemia could alter the body fat content by influencing hunger/satiety or lipid metabolism.

Food intake has been reported to be increased in diabetic rodents infused with insulin\textsuperscript{196} and to stimulate hunger in man irrespective of glucose levels.\textsuperscript{197} The relationship between insulin and food intake seems unclear, however, since it has been suggested that insulin also stimulates satiety.\textsuperscript{62}

Since insulin resistance in adolescents with type 1 diabetes is most pronounced regarding peripheral glucose uptake, another potential mechanism underlying the weight gain is that hyperinsulinaemia amplifies the insulin effect on lipid metabolism, resulting in increased fat deposition. It is possible that this mechanism is operative in well-controlled adolescents who have elevated insulin levels compared to those in healthy controls.\textsuperscript{198} In contrast, poorly controlled adolescents with type 1 diabetes have lower insulin levels than BMI-matched controls\textsuperscript{198} and show impaired suppression of lipolysis.\textsuperscript{103} Hence, this mechanism would not explain the excessive weight in poorly controlled patients. This is supported by the absence of a significant association between mean daily dosage of insulin during puberty and BMI at the age of 18 in study I and between insulin dose and gain in %BF in study IV. Similar findings have been reported previously.\textsuperscript{24,41} Interestingly the weight gain observed among adolescents on MIT in the Diabetes Control and Complications Trial (DCCT) occurred with the same daily dosage of insulin as in the conventionally treated adolescents.\textsuperscript{6}

Thus it seems that the insulin dose is of less importance for the excessive weight gain. But the number of insulin doses per day has also been found to be associated with excessive weight gain. We found in study IV that girls treated with 4 insulin doses per day gained less body fat than those on 6 daily insulin doses. In the DCCT study it was claimed that such a difference in weight gain was caused by an increased frequency of hypoglycaemia.\textsuperscript{193} It has since been shown that intensive insulin therapy can lead to improved glycaemic control without increasing the frequency of hypoglycaemia,\textsuperscript{199} and we could not find any relation between energy intake caused by hypoglycaemia and gain in %BF.

To summarise, it is clear that insulin therapy is associated with weight gain, although the reasons underlying this relation are not fully understood. It is possible that a more flexible life-style made possible by MIT might increase the energy intake, resulting in a positive energy balance.
Leptin
Leptin, a hormone produced in the adipose tissue, has been considered to be a signal to appetite centres in the hypothalamus when enough energy has been stored. In study IV we found no significant difference in leptin levels between girls with diabetes and healthy controls, which is in agreement with results of Riihimaa et al.\textsuperscript{53} but in contrast with findings of higher leptin levels in adolescent girls\textsuperscript{42, 69, 70} and boys\textsuperscript{42, 70} with type 1 diabetes compared to controls after adjustment for differences in BMI. Since insulin stimulates leptin secretion, peripheral hyperinsulinaemia in adolescents with type 1 diabetes has been suggested as the reason underlying the observed difference from healthy children. In accordance with this concept, leptin levels have been found to increase in newly diagnosed children starting on insulin therapy\textsuperscript{71} and to be associated with the insulin dosage.\textsuperscript{53, 71} However, the association between insulin dose and leptin levels is controversial.\textsuperscript{67, 69, 200, 201}

Despite a positive energy balance in obese subjects, the leptin levels are often increased. This has raised the question about leptin resistance in obesity. One possible mechanism of such resistance is an impairment of leptin transfer across the blood-brain barrier.\textsuperscript{202} There has been one study in adolescents with type 1 diabetes showing a relation between raised leptin levels and weight gain, with the inference of leptin resistance.\textsuperscript{42} However, we failed to replicate this finding in study IV. The reason for this might be that we used a different method for measuring body composition. Skinfold measurements, which were used in the study by Ahmed et al., with their lower validity and reliability compared to DXA, are less suitable for longitudinal studies. Thus it seems that leptin levels often are increased in adolescents with type 1 diabetes, although the importance for the observed weight gain needs further investigations.

Energy intake
As discussed above in this thesis, daily dosages of insulin, number of insulin doses per day and possibly leptin resistance have been found to be associated with excessive weight gain. The inference from these findings has been that increased energy intake due to a flexible life-style, frequent episodes of hypoglycaemia or deficient energy regulation is a common cause behind these associations.

In studies III and IV we found no difference between girls with type 1 diabetes and control girls in total energy intake or in the relation between total energy intake and gain in %BF. Previous studies in which the sexes have been analysed separately have shown higher total energy intake in girls with diabetes\textsuperscript{15, 36} and equal energy intake in boys in comparison with controls,\textsuperscript{203} although similar total energy intake has also been reported in comparison with controls.\textsuperscript{204-206} To my knowledge there has been no previous
longitudinal study on the effect of diet on weight gain in adolescent girls with type 1 diabetes. The reports on healthy children are inconsistent, but in a recent large longitudinal study a relation was found between caloric intake and increase in BMI. The lack of association between total energy intake and gain in %BF could be explained by the fact that underreporting of food consumption is common in adolescents and is even more pronounced in overweight subjects. This is supported by the finding that total energy intake was inversely associated with BMI SDS in controls girls. Surprisingly, in girls with diabetes there was no such association. Higher accuracy in reporting by the diabetic girls and a too small sample size could be two possible reasons for this finding.

Energy-adjusted fat intake was related to gain in %BF in both girls with diabetes and control girls. Dietary fat might influence the energy balance in several ways. The thermogenic effect of fat is lower than that of carbohydrates and especially proteins. The difference between the effect of a high fat/low carbohydrate meal and a low fat/high carbohydrate meal on TEE is small, however (2%). Secondly, it has been claimed that fat may have less effect on satiety although these findings have been questioned. Furthermore, food with a high fat content is energy-dense and it has been found that energy density is of importance for satiety and total energy intake. Thus it is most probable that the association between dietary fat and weight gain is mediated by increased total energy intake.

Studies III and IV did not contribute to the understanding of why girls with diabetes gain more body fat than healthy control girls. However, since adolescents with diabetes have higher peripheral insulin levels it could be argued that this makes them more prone to fat accumulation when their energy intake is increased. Energy intake is, however, just one side of the energy balance and it is important that energy intake be matched with energy expenditure.

Energy expenditure

The increasing prevalence of obesity among healthy children and adolescents has led to growing interest in the aetiological role of energy expenditure. The relation between activity-related energy expenditure and the development of obesity is, however, still unclear. On the other hand, a sedentary life-style, as indicated by the time spent watching TV, has been found to be associated with obesity.

Despite the relatively large number of studies dealing with physical exercise/training in adolescents with type 1 diabetes, reports on the total amount of physical activity are few and contradictory. To my knowledge, there have been no longitudinal studies concerning the relation between physical activity and development of overweight in adolescents
with type 1 diabetes.

In study III we found no difference in total amount of physical activity between adolescent girls with type 1 diabetes and healthy girls, and in study IV no relation between physical activity and gain in %BF. It might be argued that adolescents with diabetes could be less motivated to do physical activities because of fear of hypoglycaemia. However, attitudes towards physical activity have been investigated and surprisingly only 7% reported the possibility of adverse insulin reactions as barriers. In a Swedish study in 1980 the majority of adolescents with type 1 diabetes were positive towards physical exercise, although they thought that ‘really doing exercise in practice was quite another thing’.

The lack of a statistically significant difference in physical activity in our study could be due to a too small sample size. However, physical activity was examined more often during wintertime in the diabetic group and more often during late winter and spring in the controls. Physical activity varies over the seasons, with higher activity levels during springtime and summer compared to wintertime. Thus, the difference in observation period could have influenced the results and the difference in physical activity might have been smaller if the studies had been made during the same time period.

Interestingly, we found a positive relation between physical activity and gain in LBM. This is in accordance with findings of increased LBM in previous exercise training studies in obese children and with reports from cross-sectional studies. Thus, it is possible that physical activity increases not only activity-related energy expenditure but also the basal metabolic rate, mediated by increased LBM.

In study III we found no association between glycaemic control or daily dosages of insulin and amount of physical activity. Previous studies have shown conflicting results regarding the association between glycaemic control and physical activity. These discrepancies could reflect not only the different methods used to assess physical activity but also the difficulties in adjusting insulin therapy and food intake to the increased energy expenditure. On the other hand, physical activity has been associated with a lower risk for microvascular complications and exercise interventions have been shown to increase insulin sensitivity and improve the lipid profile.

To summarise, there seems to be no difference in the amount of physical activity between adolescent girls with type 1 diabetes and healthy controls, although more studies are needed to confirm these findings. Since physical activity appears to have several beneficial effects on individuals with type 1 diabetes, it is of great importance to promote such activity.
Body composition

Body mass index is a well-established measure of relative fatness in adolescents and adults. This measure has been validated against body fat measured by DXA in children and adolescents, explaining 72–92% of the variance in total body fat and 63–79% of the variance in percentage body fat.47, 236, 237 Hence, BMI is an acceptable measure for comparing body fatness between groups. Accordingly, the finding in study I that adolescent girls with type 1 diabetes had a higher BMI than control girls can be considered to reflect a difference in body fatness.

Despite the high correlation between BMI and %BF, the variation in percentage body fat is large for a given BMI value, making BMI unsuitable for comparing body composition between individuals and small groups. Moreover, height, sex and age alter the relation between BMI and fatness.237 Shorter individuals have lower BMI, girls have higher %BF for a given BMI,236 and increased LBM as a result of training will give a higher BMI.

Nevertheless, the finding of a higher BMI, implying higher %BF, in girls with type 1 diabetes in study I was supported in study II. It was found there that %BF estimated from skinfold measurements was higher in girls with diabetes, which is in agreement with previous studies on adolescents with type 1 diabetes (see Table 1).

Skinfold measurements are a traditional method for assessing body composition that are non-invasive and inexpensive. They have several drawbacks, however. The skinfold measurement is difficult to assess. The CV for triceps skinfold measured by the same investigator in one day is 7% and that for subscapular skinfold is 4%.238

Estimations of %BF from skinfolds is based on the relation between skinfolds and body density and involves several assumptions. A constant density of LBM and a constant relation between subcutaneous fat and FM are assumed, making it important to use a population-specific equation to calculate FM or %BF. Several skinfold equations have been developed for healthy adolescents and young adults.181, 239-241 In the present study we used the equation proposed by Slaughter et al.,181 as it was developed from an appropriate population and has been found to be the most valid skinfold equation.242

When Slaughter’s equation was validated against a 4-compartment (4-C) model the mean difference in %BF between skinfold measurement and the 4-C model was found to be -3.5 – 0.2 % units and with a wide limit of agreement (± 2 SD for difference) between ± 8–10% units.238, 242, 243 Furthermore, the bias was found to increase with increasing fatness.243 These limitations make the skinfold method unsuitable for longitudinal studies of changes in body composition.

In study II we also used dual-energy X-ray absorptiometry, which is considered to be a more valid technique for assessment of body composition.
composition. In accordance with the results of skinfold measurements, we found higher %BF in girls with diabetes compared to control girls. To my knowledge our study is the first one in which this method has been used in an adolescent population with type 1 diabetes.

In study II we found that the mean difference in %BF between skinfold and DXA was -3% units, with limits of agreements at ±7% units in control girls. This finding is comparable with that in the validation studies, where skinfold measurements underestimated %BF. In girls with diabetes the picture was different. Skinfold measurements overestimated %BF and the limits of agreements were wider, ±9%. Most importantly, there was a relation between increasing fatness and the difference between the two methods.

One possible explanation for this could be increased stiffness in subcutaneous fat caused by glycated collagen. Interestingly, Tillman et al. found that on comparison with healthy adolescents, despite the same levels of BMI both girls and boys with diabetes had significantly thicker triceps and biceps skinfold. Thus, it seems to be of importance to develop a skinfold equation from a population of adolescents with type 1 diabetes to increase the validity, or preferably to use more valid body composition techniques if available.

Associations between body fat and glycaemic control

Obesity is associated with insulin resistance in adults and healthy adolescents and adolescents with type 1 diabetes. In particular the visceral fat depot is of importance for mediating the reduced sensitivity to insulin in adults, but there have been similar reports during adolescence. The importance of the visceral fat deposits is supported by the finding in study V that insulin sensitivity was negatively related to W/H ratio but not to BMI SDS.

Glycaemic control deteriorates during puberty in both sexes, but most markedly in girls. This possibly reflects the reduced insulin sensitivity caused by increased GH levels during puberty, although psychological or behavioural factors are of clear importance. However, the difference in body composition, with higher fat deposits found in girls, might also influence insulin sensitivity. Increased BMI, an indirect estimated of body fat, during adolescence in type 1 diabetes has been found to be associated both with good and with deteriorated glycaemic control.

In study II we found that abdominal fat (visceral + subcutaneous) and a centralised fat distribution were strongly associated with both glycaemic control and daily dosage of insulin. We did not examine visceral fat directly, but DXA-derived measurements of abdominal fat have been found to correlate with CT measurements of visceral fat. These associations could
be explained by increased FFA flux from an enlarged visceral fat deposit.

The physiology of visceral fat differs from that of subcutaneous fat. FFA release from visceral fat is higher than that from subcutaneous fat, where insulin antilipolytic effects are more pronounced. Furthermore, FFA from visceral fat drains directly to the portal vein giving high concentrations to the liver and leading to increased gluconeogenesis and triglyceride synthesis. Raised FFA levels impair insulin actions in the liver, but also influence peripheral insulin sensitivity. FFA has been associated with impaired intracellular insulin signalling, resulting in reduced GLUT-4 translocation in muscle. It is also possible, although not proved, that elevated FFA levels increase intramyocellular lipid contents, which has been associated with insulin resistance. Of interest in this context is the previous finding that suppression of lipolysis is impaired in adolescents with poorly controlled type 1 diabetes and is inversely related to glucose uptake.

Adipocytes also produce hormones (adipocytokines) that affect insulin sensitivity. Tumor necrosis factor (TNF)-α is produced in adipose tissue and is correlated with insulin sensitivity in obese adults. TNF-α is primarily of importance for adipocytes, where it stimulates lipolysis and inhibits lipogenesis by impairing insulin signalling. Its effect on peripheral insulin sensitivity is probably mediated by increased FFA levels.

Another candidate adipocytokine is adiponectin, which is reduced in obese adolescents and has been found to be positively associated with insulin sensitivity. Adiponectin is thought to enhance fat oxidation in muscle and reduce glucose production in the liver. However, there has been one report on patients with type 1 diabetes in whom the level of adiponectin was elevated. Thus, the importance of adiponectin in type 1 diabetic patients is still unclear.

In conclusion, increased adipose tissue, especially visceral fat, is of importance for peripheral insulin sensitivity in adolescents with type 1 diabetes presumably mediated by increased FFA levels.

**Lipids**

In study II we found that the total cholesterol level was higher in the girls with type 1 diabetes than in the control girls whereas there was no significant difference in the triglyceride level. Elevated total cholesterol levels have previously been noted in children and adolescents with type 1 diabetes compared to healthy controls, while the triglyceride levels have been reported to be raised, similar or even decreased. In accordance with previous studies, we also found a positive relation between HbA1c and total cholesterol and triglycerides. A centralised fat distribution was positively related to the total
cholesterol and triglyceride levels in adolescent girls with type 1 diabetes. Obesity, especially with a centralised fat distribution, has been found to be associated with dyslipidaemia in healthy children and adolescents\textsuperscript{270} and obese adolescents girls\textsuperscript{272}. Triglycerides have also been reported to be associated with the W/H ratio\textsuperscript{273} and intraabdominal fat\textsuperscript{274} in adults and triceps skinfold in children\textsuperscript{265} with type 1 diabetes.

The association between glycaemic control, a centralised fat distribution and dyslipidaemia might be explained by a diminished antilipolytic effect of insulin, with increased FFA flux from visceral fat deposits, which would increase hepatic triglyceride production\textsuperscript{275}. Furthermore, clearance of lipoprotein might be delayed as a result of decreased activity of lipoprotein lipase. However, there is one report of a normal chylomicron clearance rate in adolescents with type 1 diabetes\textsuperscript{268}, which makes this mechanism less likely. It is also possible that differences in dietary fat intake\textsuperscript{269}, activity of cholesteryl ester transfer protein\textsuperscript{266, 276} and hepatic lipase\textsuperscript{274} may influence the cholesterol and triglyceride levels in diabetic patients.

**Intervention with metformin**

In study V we found that addition of metformin to intensive insulin therapy improved glycaemic control (HbA\textsubscript{1c}) in adolescents with poorly controlled type 1 diabetes. This finding was confirmed in a recently published study on adolescents with type 1 diabetes, who also showed a reduction in fasting glucose levels\textsuperscript{277}.

In patients with type 2 diabetes the effect of metformin on glucose metabolism is mainly mediated by a reduction of hepatic glucose production and to a lesser degree by an increase in glucose uptake. However, it has also been suggested that metformin reduces lipolysis in obese patients\textsuperscript{175}. The mechanism underlying the improvement of insulin sensitivity caused by metformin has been less well studied in patients with type 1 diabetes. We found that glucose uptake as measured by the euglycaemic hyperinsulinaemic clamp technique was increased during metformin therapy. Since reduced glucose uptake is the main feature of insulin resistance during puberty, it seems possible that this mechanism is the most important for the improved glycaemic control. However, influences on lipid metabolism could also be of importance. Interestingly, Hamilton et al.\textsuperscript{277} found reduced fasting glucose levels in adolescents treated with metformin, indicating that hepatic glucose production was reduced.

The daily dosage of insulin was not decreased during metformin treatment in our study. This is in contrast to previous findings in adult and to the report by Hamilton et al. of a 10\% reduction in insulin requirements. The absence of a significant reduction of the insulin dose in our study could be explained by the fact that the glycaemic control, although improved
by metformin, was still impaired, indicating hypoinsulinaemia. The shortcomings of self-reported insulin doses are another possible cause of the conflicting results, since insulin omission is common in adolescents with diabetes and has been found to be increased with deteriorated glycaemic control.129

Both the study by Hamilton et al. and our study were conducted over a three-month period. In two other previous studies on adolescents with type 1 diabetes conducted over six months no significant effect of metformin was found at closure of the study.164, 165 Interestingly, Walravens et al.164 reported a positive effect at three months that could not be demonstrated after six months. These findings might be explained by poor compliance, possibly caused by gastrointestinal side effects. However, in study V and in the study by Hamilton et al. we found no increased frequency of side effects in the metformin group compared the placebo-treated patients.

Thus, metformin as additional treatment to insulin improves glycaemic control, possibly by increasing peripheral glucose uptake. However, the long-term efficacy has been questioned and needs to be studied further.

Future perspectives

We found in studies I and II that BMI and %BF were increased in diabetic girls during late puberty, but also that the difference in body mass index decreased between the ages of 18 and 22 in girls. Prospective studies on the change in body composition from late adolescence to young adulthood are needed in order to confirm the hypothesis that differences in body fat between diabetic and healthy adolescents decrease during the transition to adulthood.

Valid methods are necessary when examining longitudinal changes in body composition. Skinfold measurements are inexpensive and suitable for studies with large populations. We found, however, that this technique is less valid than DXA in girls with type 1 diabetes. Thus, in order to improve the validity of estimates of percentage body fat from skinfold measurements, new population-specific equations need to be developed.

A centralised fat distribution was found in study II to be associated with poor glycaemic control and a disturbed lipid profile. The mechanisms underlying these findings could not be elucidated from our present study. Studies of potential mechanisms such as increased visceral fat deposits with high FFA turnover or altered levels of adipocytokines would enhance our understanding and possibly provide clues for new treatment strategies.

Thiazolidinediones regulate lipid metabolism and it has been suggested that they interfere with TNF- activity. TNF- is produced in adipose tissue and diminishes insulin sensitivity by increasing lipolysis. It is possible that an increased body fat mass in adolescents with type 1 diabetes will result
in increased TNF- levels and reduced insulin sensitivity. Thus it would be interesting to examine the effect of thiazolidinediones on glycaemic control and insulin sensitivity in adolescents with type 1 diabetes.

Previous observations, including our own, indicate that metformin as an addition to intensive insulin therapy improves glycaemic control in adolescents with poorly controlled type 1 diabetes, although the long-term efficacy has been questioned. Most studies have been conducted with small sample sizes and over relatively short time periods. Consequently, clinical studies covering longer time periods are needed to examine the long-term effects. Additionally, the mechanism by which metformin improves glycaemic control and insulin sensitivity in adolescents with type 1 diabetes needs further investigation. Since lipolysis is increased in patients with poorly controlled type 1 diabetes and metformin seems to enhance the insulin action on lipolysis, investigations of the lipid metabolism in such patients would be of specific importance.
Conclusions

What was already known on this topic?
- Excessive weight gain is common among adolescent girls with type 1 diabetes. Previous studies have shown a higher body mass index and higher skinfold measurements in girls with diabetes compared to healthy controls.
- Excessive weight gain is caused by positive energy balance. Reports on energy intake and energy expenditure in adolescents with type 1 diabetes are few and there has been no prospective study addressing the effects on body composition.
- Studies regarding the effect of metformin in adolescents with type 1 diabetes are few and inconclusive.

What this thesis adds
- Body mass index, at the age of 18, in girls with type 1 diabetes is associated with poor glycaemic control but not with daily dosage of insulin during puberty.
- Body fat content as measured by DXA is higher in girls with type 1 diabetes than in healthy control girls in late puberty.
- Skinfold measurements overestimate percentage body fat in adolescent girls with type 1 diabetes.
- Fat distribution in adolescent girls with type 1 diabetes is associated with glycaemic control and lipid levels.
- There is no significant difference in either total energy intake or physical activity between girls with type 1 diabetes and control girls.
- Dietary fat intake predicts gain in body fat in girls with type 1 diabetes.
- Physical activity predicts gain in lean body mass but not changes in body fat.
- Addition of metformin to intensive insulin therapy improves glycaemic control and increases peripheral insulin sensitivity.
Clinical implications
• Metformin is valuable as an addition to intensive insulin therapy when
glycaemic control deteriorates in adolescents with type 1 diabetes.
• Increased dietary advice is necessary to prevent excessive weight gain.
• Physical activity should be promoted in order to increase lean body
mass.
• Existing skinfold equations for estimating percentage body fat are not
useful in adolescent girls with type 1 diabetes.
Summary in Swedish
(svensk sammanfattning)

Bakgrund - Ungdomar med typ 1 diabetes ökar ofta mer i vikt under puberteten jämfört med friska kontroller, ett problem som är mer uttalat hos flickor. Tidigare studier har funnit högre vikt och kroppsmasseindex (BMI) hos flickor med typ 1 diabetes under den senare delen av puberteten i jämförelse med friska flickor. Intensiv insulin behandling har föreslagits som en tänkbar orsak bakom dennaviktskillnad men i övrigt är orsakssambanden ofullständigt kända. Oavsett bakomliggande orsak är det viktigt att motverka överviktsutveckling hos ungdomar med diabetes eftersom ett högt BMI kan försämra känsligheten för insulin och ge en försämrad metabol kontroll med ökad risk för framtida komplikationer.

Målsättning - Syftet med avhandlingen var att närmare studera kroppssammansättningen hos flickor med typ 1 diabetes i jämförelse med friska flickor samt dess relation till fysisk aktivitet och energiintag. Vi undersökte också om tilläggsbehandling med metformin, ett läkemedel som ökar insulinkänsligheten, förbättrar metabol kontroll och insulinkänslighet hos ungdomar med dåligt reglerad typ 1 diabetes.

Resultat - BMI och andelen kroppsfett var högre hos flickor med typ 1 diabetes i jämförelse med friska flickor och en fördelning av fetmassan till bukregionen var kopplad till försämrad metabol kontroll och förhöjda blodfletter. Flickor med diabetes var lika fysiskt aktiva och hade lika stort energiintag som friska flickor. Däremot fann vi att ett stort intag av fet mat förhöjde risken att få en ökning av den procentuella andelen fettväv både hos friska flickor och hos flickor med diabetes. Tilläggsbehandling med metformin under tre månader förbättrade den metabola kontrollen och ökade känsligheten för insulin.

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