Muscle Fiber Composition and Capillary Density in Turner Syndrome

Evidence of increased muscle fiber size related to insulin resistance

Claus Højbjerg Gravholt1 Birgit Nyholm1 OLE SCHMITZ1 Bengt Saltin2 Jens Sandahl Christiansen1

OBJECTIVE — To assess muscle fiber composition and capillary density in Turner syndrome, a condition linked with insulin resistance and increased frequency of type 2 diabetes, and link these findings with insulin sensitivity and physical fitness.

RESEARCH DESIGN AND METHODS — A total of 10 patients with Turner syndrome who were off hormone replacement therapy (aged 32.7 ± 8.9 years) and a control group of 14 normal women (aged 35.6 ± 9.3 years) were studied. None of the participants had diabetes or any family history of type 2 diabetes. An oral glucose tolerance test was performed, and insulin sensitivity was assessed by homeostasis model assessment (HOMA) and a composite whole-body insulin sensitivity index (ISIcomp). Physical fitness was assessed, and a muscle biopsy was obtained.

RESULTS — Women with Turner syndrome were insulin resistant, as seen by a lower ISIcomp (P = 0.003) and increased glucose (P < 0.0005) and insulin (P = 0.01) levels at 120 min. Impaired glucose tolerance was present in most Turner syndrome patients (6 of 10), but not in the control subjects. Women with Turner syndrome had an increased size of type Ila fibers (P = 0.01), whereas the size of their type I and Ila fibers were comparable with the control group. The groups did not differ in percentage of type I, Iia, or IIX fibers, and there was no difference in the capillary density. Significant correlations were found among ISIcomp, the HOMA index (R_HOMA), and the mean area of type Ila fibers (ISIcomp: r = -0.632, P = 0.002; R_HOMA: r = 0.570, P = 0.006). Furthermore, capillaries/type Ila fibers correlated significantly with ISIcomp (r = -0.618, P = 0.01). There were no significant correlations between VO2max and muscle fiber composition.

CONCLUSIONS — Healthy women with Turner syndrome are characterized by impaired glucose tolerance, insulin resistance, low physical capacity, and enlarged type Ila muscle fibers, indicating diminished oxygen and substrate supply for metabolic processes. These findings could be indicative of a prediabetic state.

Diabetes Care 24:1668–1673, 2001

Turner syndrome is a condition involving total or partial absence of one X chromosome in all or part of the body’s cells, reduced final height, absence of female sex hormones, reduced amounts of male sex hormones (1), and infertility in most cases. Abnormal glucose tolerance, hyperinsulinemia, and reduced insulin sensitivity is found with increased frequency in adolescents with Turner syndrome (2–6) and in adults (7,8). We have recently found increased mortality in Turner syndrome, where diabetes was reported as an underlying cause of death in 25% of the cases (9), as well as increased morbidity with increased frequency of ischemic heart diseases, hypertension, and type 2 diabetes (10). Thus, women with Turner syndrome are prone to develop facets of the metabolic syndrome (syndrome X), in which hypertension, dyslipidemia (high triglycerides and low HDL cholesterol), type 2 diabetes, obesity, hyperinsulinemia, and hyperuricemia are all seen as different manifestations of the same disease (11).

Skeletal muscle composition and capillary density is associated with insulin sensitivity (12) and physical fitness (13). Previously, we have shown that healthy offspring of patients with type 2 diabetes, who are at increased risk of developing diabetes themselves, have an increased number of type IIX fibers, which may indicate a reduced oxidative capacity (13). It has been shown that physical exercise is capable of transforming type IIX fibers to type Ila fibers, possibly postponing the advent of type 2 diabetes and thus playing a role in the treatment of type 2 diabetes (14). Postmenopausal women with impaired glucose tolerance have larger type Ila and IIX muscle fibers compared with women with normal glucose tolerance (15). The aim of the present study was to determine whether women with Turner syndrome have an altered muscle fiber composition and capillarization. To that end, we examined muscle biopsies from adult women with Turner syndrome and compared them with biopsies from a group of normal women. We assessed insulin sensitivity and physical fitness and performed an oral glucose tolerance test (OGTT). The study was performed as a part of a clinical trial investigating the carbohydrate and lipid metabolism in adult Turner syndrome (8,16).
Table 1—Anthropometric data and lipid variables in Turner syndrome patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Turner syndrome</th>
<th>Control subjects</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.7 ± 8.9</td>
<td>35.6 ± 9.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.7 ± 8.5</td>
<td>161.1 ± 5.3</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.2 ± 11.8</td>
<td>68.4 ± 9.4</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.9</td>
<td>24.8 ± 2.9</td>
<td>0.96</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.84 ± 0.08</td>
<td>0.81 ± 0.05</td>
<td>0.3</td>
</tr>
<tr>
<td>LBM (%)</td>
<td>69.3 ± 4.1</td>
<td>72.8 ± 6.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.3 ± 1.1</td>
<td>4.5 ± 0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>0.2†</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.78 ± 0.45</td>
<td>1.04 ± 0.41</td>
<td>0.2</td>
</tr>
<tr>
<td>FFAs (mmol/l)</td>
<td>0.95 ± 0.30</td>
<td>0.52 ± 0.20</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

*Student’s t test unless otherwise indicated. †Mann-Whitney U test.

RESEARCH DESIGN AND METHODS — The study group consisted of 10 patients with Turner syndrome, verified after karyotyping: 45,X (n = 5); 45,X/46,X,i(Xq) (n = 2); 46,X,del(Xq) (n = 1); 45,X/46,X,idic(X) (n = 1); and 45,X/46,XY (n = 1). There was also a control group of 14 normal women with a presumed normal karyotype. Both groups had no evidence of diabetes or any family history of type 2 diabetes. Two participants (the youngest) had received growth hormone during their adolescence, whereas none had received oxandrolone. Exclusion criteria were untreated clinical hypo- or hyperthyreosis, former or present malignant disease, clinical liver disease, family history of thromboembolic diseases, extreme obesity (BMI >40), clinical heart disease, other significant diseases; allergy toward any of the drugs used, and heavy smoking (20 cigarettes/day). All women examined were clinically euthyroid. None of the women were smokers, although two of the control subjects smoked. The control group was matched with respect to age and BMI (Table 1).

Before the study began, all subjects received oral and written information concerning the study before giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee and the Health Board under the Ministry of Health. The participants with Turner syndrome were studied after a 4-month washout period, during which the participants were not allowed to take their usual hormone substitution. All of the women with Turner syndrome had received regular hormonal replacement therapy before entering the study, consisting of 17β-estradiol (2 mg) with either norethisterone (1 mg) or medroxyprogesterone (5 mg).

Experimental procedure
All subjects were studied on two separate days. All studies started at 8:00 A.M. in the Clinical Research Center, Medical Department M, University Hospital of Aarhus, the morning after an overnight fast (10–12 h). For at least 3 days before the study, the subjects consumed carbohydrate-rich diets with at least 300 g carbohydrate/day. Participants were asked to not perform major physical exercise for the last 3 days before examination.

Body composition. After an initial bed rest of at least 45 min, fat free mass was determined using bioelectrical impedance (Animeter; HTS-Engineering, Odense, Denmark) (17). BMI was calculated as weight (kilograms) divided by height (meters) squared.

OGTT. An OGTT was performed with baseline samples of glucose and insulin. Glucose (75 g) was administered orally, and subsequent samples were drawn at 30, 60, 90, and 120 min.

Insulin sensitivity. Insulin sensitivity was calculated using the homeostasis model assessment (HOMA) index (R_{HOMA}) (18) and the composite whole-body insulin sensitivity index (ISI_{comp}) during the OGTT (19). The R_{HOMA}, which is based on simultaneous sampled fasting values of insulin and glucose, has been previously shown to correlate well with the euglycemic-hyperinsulinemic clamp in the assessment of insulin resistance in both normal and diabetic subjects (18), and it may be thought of as primarily illustrating hepatic insulin sensitivity (19). The R_{HOMA} is calculated as follows:

\[
R = \text{fasting insulin/22.5} \times e^{-\text{ln fasting glucose}}
\]

The ISI_{comp} is a composite measure of whole-body insulin sensitivity that includes components of both hepatic and peripheral tissues and uses both fasting and stimulated (during an OGTT) values of glucose and insulin. It is calculated as follows:

\[
\text{ISI}_{\text{comp}} = 10.000/\sqrt{(\text{FPG} \times \text{FSI})} \times \left(\text{mean OGTT glucose concentration} \times \text{mean OGTT insulin concentration}\right)
\]

where FPG denotes fasting plasma glucose and FSI denotes fasting serum insulin. This measure has recently been validated in subjects with normal, impaired, and diabetic glucose tolerance using the euglycemic-hyperinsulinemic clamp as the gold standard and has been found to be superior to other more crude measures of insulin sensitivity, such as R_{HOMA} (19).

Physical fitness (V_{O2max}). A 6-min submaximal exercise test with continuous monitoring of heart rate was performed on a bicycle ergometer (Monark Ergometric 829 E; Monark Exercise, Varberg, Sweden) using a workload of 300–1,500 kpm/min, depending on age and reported physical activity by the subject. The mean heart rate during the last 2 min of work (>120 beats/min) was used for calculation of the maximal aerobic capacity (V_{O2max}) (20). In our study, the day-to-day intra-individual coefficient of variation (CV) was 9% (21). This indirect measure of maximal aerobic capacity has been shown to correlate well with a direct measure of maximal aerobic capacity, with a CV of <10% (22,23).

Muscle biopsy procedure. The subjects rested in the supine position for half an hour after the OGTT. Local anesthesia (between 5 and 10 ml lidocaine 1%) was applied —5–10 min before the biopsy. A muscle biopsy was then obtained from the vastus lateralis 15–20 cm above the knee, using a Bergstrom needle (4.5–5.0 mm external diameter). Part of the biopsy was examined under a magnifying glass to determine fiber orientation and then

Gravholt and Associates
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Table 2—Data from the OGTT and VO_{2max} in Turner syndrome patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Turner syndrome</th>
<th>Control subjects</th>
<th>P*</th>
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<tbody>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>5.2 ± 0.8</td>
<td>4.8 ± 0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>120-min</td>
<td>7.6 ± 1.3</td>
<td>5.1 ± 0.9</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>AUC (mmol/l · 120 min)</td>
<td>1,039 ± 142</td>
<td>751 ± 141</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>37 ± 18</td>
<td>26 ± 18</td>
<td>0.2†</td>
</tr>
<tr>
<td>120-min</td>
<td>262 ± 148</td>
<td>117 ± 65</td>
<td>0.01†</td>
</tr>
<tr>
<td>AUC (pmol/l · 120 min)</td>
<td>32,963 ± 14,982</td>
<td>21,253 ± 10,400</td>
<td>0.06†</td>
</tr>
<tr>
<td>ISI_{comp}</td>
<td>17.1 ± 11.9</td>
<td>46.8 ± 39.6</td>
<td>0.003†</td>
</tr>
<tr>
<td>HOMA</td>
<td>8.9 ± 5.6</td>
<td>5.6 ± 4.1</td>
<td>0.1</td>
</tr>
<tr>
<td>VO_{2max} (ml O_{2}/min · kg)</td>
<td>35.6 ± 10.0</td>
<td>41.6 ± 7.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Student's t test unless otherwise indicated. †Mann-Whitney U test. AUC, area under the curve.

mounted in an embedding matrix (OCT compound; Miles, Elkhart, IN) and frozen in isopentane (Bie and Berntsen, Højbjerg, Denmark), cooled to its freezing point with liquid N₂. The other part of the biopsy was frozen immediately in liquid N₂. The final frozen specimens were kept at −80°C until analysis.

**Histochemical staining.** Conventional amylase-PAS staining as described by Andersen (24) and Andersen and Kroese (25) was performed to stain capillaries. Myofibrillar ATPase staining with preincubations of pH 10.3, 4.6–4.8, and 4.37 was used to identify muscle fiber types (25a). Computer image analysis was performed using a TEMA image analysis system (Scan Beam, Hadsund, Denmark). The fibers of the vastus lateralis specimen were classified as type I, type IIa, and type IIb. This latter subgroup of the type II fibers may be renamed type IIX because recent studies have revealed that it most closely corresponds to the type IIX fiber in the rat (26). It is doubtful whether the type IIb fiber of the rat can be found in skeletal muscle of humans. In both groups, a very small number of fibers (<1%) were stained with all pH preincubations. These fibers are coexpressing myosin heavy chain–I/IIa (27). For the classification of fibers, as many distinct fibers as possible were counted (≥300). An area (one area or two to three areas together) containing ≥200 fibers in each section was selected for the capillary counting and fiber area measurement. These demands could only be fulfilled in 5 of the patients with Turner syndrome and in 11 of the control subjects. Analysis of capillary profiles included the capillary-to-fiber ratio, capillaries per square millimeter of muscle tissue, and number of capillaries around each fiber type. The number of capillaries related to each fiber type was determined from 20 fibers of each fiber type. All transversely cut capillaries were counted. If a capillary was sectioned longitudinally, it was counted as one each time it crossed three or more muscle fibers. The CV was as follows in our laboratory: fiber types, 5–10%; fiber size, 3–5%; capillaries, 3–6%.

**Analytical methods.** Plasma glucose was measured immediately in duplicate on an autoanalyzer (Beckman Instruments, Palo Alto, CA) by the glucose oxidase method. Insulin was measured by enzyme-linked immunosorbent assay using a two-site immunoassay, which does not detect proinsulin (split(32-33)- and des(31-32)-proinsulin) whereas split(65-66)- and des(64-64)-proinsulin cross-react 30 and 63%, respectively. The intraassay CV was 2.0% at a serum level of 200 pmol/l (28). The level of serum free fatty acids (FFAs) was determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Cholesterol and other measures of lipid metabolism were determined on a COBASE Integra (Roche, Hvidovre, Denmark).

**Statistical analysis**

Data were examined by Student’s two-tailed unpaired t test or Mann-Whitney U test, Pearson or Spearman correlation, together with linear and multiple linear regression. Results are expressed as the mean ± SD. Areas under curves were calculated using the trapezoidal rule. Significance levels <5% were considered significant; for the correlation analyses, a protected significance level of 1% was considered significant. All statistical calculations were done with SPSS for Windows version 10.0 (SPSS, Chicago, IL) on a Pentium PC.

**RESULTS**

**Body composition**

The two groups were well matched for age and BMI; however, lean body mass (LBM) was higher in control subjects, though not reaching statistical significance. This phenomenon of diminished LBM, despite similar BMI, in subjects with Turner syndrome has been described before (29). Values for height and weight were lower, as expected, in women with Turner syndrome (Table 1).

**Insulin sensitivity, OGTT, and physical capacity**

Fasting plasma glucose and serum insulin was comparable in the two groups, as was an index of hepatic insulin sensitivity, the R_{HOMA} (P = 0.11) (Table 2). However, both the ISI_{comp} index of insulin sensitivity (P = 0.003) and the glucose (P < 0.0005) and insulin (P = 0.01) level at 120 min was increased in women with Turner syndrome, indicating insulin resistance. After the OGTT, all subjects were categorized according to American Diabetes Association criteria, and impaired glucose tolerance was present in 6 of 10 women with Turner syndrome but in none of the control subjects. Impaired fasting glucose (fasting glucose >6.1 mmol/l and <7.0 mmol/l) was present in 1 of 10 women with Turner syndrome and in none of the control subjects. Serum lipids were comparable in the two groups, except FFA, which was increased in women with Turner syndrome (Table 1). VO_{2max} tended to be reduced (by 15%) in women with Turner syndrome, the difference not reaching statistical significance (P = 0.1).

**Muscle fiber composition and capillary density**

Women with Turner syndrome had an increased size of type Ila fibers (Turner versus control subjects: 5,584 ± 326 vs. 4,242 ± 333 μm², P = 0.01), whereas the size of type I (4,735 ± 435 vs. 4,699 ± 360 μm², P = 0.9) and IIX (3,809 ± 239...
vs. 3,304 ± 402 μm², P = 0.3) fibers were comparable to the control group. The increased size of the type IIa fibers was explained by differences in HOMA or ISI_comp in multiple regression analysis, whereas status (i.e., being a woman with Turner syndrome or a control subject) or indexes of body composition did not explain the difference (Fig. 1). Furthermore, the groups did not differ in number of type I (41.6 ± 3.5 vs. 43.1 ± 3.0%, P = 0.7), IIa (30.7 ± 3.0 vs. 33.8 ± 3.7%, P = 0.5), or IIx (27.1 ± 2.0 vs. 22.9 ± 2.8%, P = 0.3) fibers, nor did they differ when assessed as area of fiber (%) (data not shown). There was no difference in the capillary density (expressed as capillaries per fiber type, capillaries per fiber, or capillaries per millimeter squared) between the two groups (data not shown).

**Conclusions**

The main finding of the present study is that healthy women with Turner syndrome and a normal fasting glucose, but who have reduced insulin sensitivity as assessed by the ISI_comp, have an increased size of type IIa fibers in their muscle fiber composition when compared with age- and BMI-matched control subjects. All other indexes of muscle fiber composition and capillarization were comparable with a matched control group.

Women with Turner syndrome have increased mortality, with diabetes mellitus as an underlying cause of death in 25% of the cases (9). In an epidemiological study of the entire population of women with Turner syndrome in Denmark, we found that type 2 diabetes occurred with an increased relative risk of 4, and that the risk of type 1 diabetes was also increased (RR = 11) (10). In earlier studies, we and others have described increased 120-min values of glucose and insulin during an OGTT in a large group of women with Turner syndrome (n = 27), whereas the insulin sensitivity index derived from the Minimal Model (30) was comparable with control subjects (7,8). Likewise, we found increased 24-h systolic and diastolic blood pressure, increased night-to-day ratio, and decreased maximal oxygen uptake (8). Earlier, Caprio et al. (6) studied young girls and adolescents with Turner syndrome, using
the euglycemic-hyperinsulinemic clamp and indirect calorimetry, and found decreased nonoxidative glucose disposal, suggesting an early metabolic defect, perhaps intracellularly, in glucose metabolism. In the present study, insulin resistance was present (i.e., reduced ISI-comp., increased 120-min value of glucose and insulin, increased level of FFAs, and a decreased maximal oxygen uptake), whereas $R_{HOMA}$, fasting glucose, and insulin were comparable to values in control subjects. Our finding of increased size of type IIA fibers in women with Turner syndrome and impaired glucose tolerance is in accordance with findings in postmenopausal female subjects. Women who are postmenopausal and who have impaired glucose tolerance have larger type IIA and IIX muscle fibers compared with women with normal glucose tolerance (15). Furthermore, the correlation between insulin sensitivity and the size of type IIa fibers, as seen in the present study, has also been shown in postmenopausal women (15). Female subjects who are postmenopausal and who have impaired glucose tolerance have been characterized with low levels of sex-hormone binding-globulin and high androgens (31), a situation that is not present in Turner syndrome, because they are characterized by low levels of androgens, with no change or even a reduction in levels of androgen during hormone replacement therapy (HRT) (1). In the present study, we have not been able to study the effects of HRT, if any, on muscle morphology. However, this would be of considerable interest because of the observation that a progressive decline in muscle force is normally seen in postmenopausal women (32), and that this decline can be prevented by sex-hormone replacement therapy (33), perhaps indicating some effects of HRT on muscle morphology. In healthy relatives of type 2 diabetic patients, an increased number of type IIX fibers has been found, and tight correlations between maximal oxygen uptake and capillarization is present (13); however, the lack of such an association in the present population may again be caused by a type 2 error. In male subjects with impaired glucose tolerance, increased capillarization, especially around type IIX fibers, has been shown to be predictive of the development of type 2 diabetes during a 15-year period (34). In addition, the low maximal oxygen uptake that characterizes healthy relatives of type 2 diabetic patients, in comparison with well-matched control subjects, also seems to be a characteristic of healthy women with Turner syndrome, as seen in our data and in a previous study (8). Thus, several indexes point toward Turner syndrome as a condition that can be characterized as prediabetic. Interestingly, however, lipid metabolism does seem to be normal in Turner syndrome, despite other components of the metabolic syndrome being present. We found normal levels of lipid parameters in the current study, as was also shown previously (16).

In conclusion, healthy women with Turner syndrome are characterized by impaired glucose tolerance, insulin resistance, low physical capacity, and enlarged type IIA muscle fibers, indicating diminished oxygen and substrate supply for metabolic processes in accordance with data from other studies. However, lipid parameters were not elevated. Taken together, these findings could be indicative of a prediabetic state.

Acknowledgments — C.H.G. received financial support through a research fellowship from the University of Aarhus. This study was supported by a grant from the Danish Diabetes Association and the Danish Health Research Council (grant no. 9600822, Aarhus University-Novo Nordisk Center for Research in Growth and Regeneration).

We thank Lone Korsgaard and Eva Sejer Christoffersen for their expert technical help.

References


33. Phillips SK, Rook KM, Siddle NC, Bruce SA, Woledge RC: Muscle weakness in women occurs at an earlier age than in men, but strength is preserved by hormone replacement therapy. *Clin Sci Colch* 84:93–98, 1993