Improvement of Insulin Resistance in Diet-Induced Obese Mice by Sulodexide, an Endothelial Glycocalyx Mimetic

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Abstract

Recently, we showed that the induction of peripheral insulin resistance in diet-induced obese mice was accompanied by impaired barrier properties of the endothelial glycocalyx in the microcirculation of hindlimb muscle. We hypothesized in the current study that sulodexide, an endothelial glycocalyx mimetic, would improve insulin-mediated glucose disposal in high-fat diet (HFD) fed mice.

Mice received a HFD for 6 or 18 weeks with or without therapy. Therapy consisted of sulodexide (0.15 mg/mL) or, as a reference, metformin (0.3 mg/mL) in the drinking water in the last two weeks of the HFD feeding period. To evaluate insulin action, mice were anesthetized; subsequently, they received a bolus of 1g/kg glucose via an i.p. cannula (IPGTT) while glucose and insulin levels were monitored for 120 and 90 minutes, respectively.

Two weeks of sulodexide treatment in the 6 weeks HFD fed mice was associated with significantly lower glucose levels measured during the IPGTT without a significant change in insulin levels. After a HFD for 18 weeks, sulodexide treatment did not significantly reduce glucose levels during the IPGTT yet insulin resistance appeared to be improved, as reflected by a significant decrease of the product of the area under the curve for both the glucose and insulin levels. Two weeks of metformin treatment did not affect glucose tolerance in the 6 weeks HFD fed mice. Metformin did also not affect glucose and insulin levels after 18 weeks of HFD.

Oral sulodexide treatment for 2 weeks is able to improve insulin-mediated glucose disposal in diet-induced obese mice. Our data indicate that timely treatment with the glycocalyx-mimetic sulodexide can be a potential therapy to alleviate insulin resistance and prevent the development of glucose intolerance and type II diabetes in obesity.

INTRODUCTION

Obesity is a fast growing health-related problem, and is associated with a major risk for developing insulin resistance. Insulin resistance refers to a decreased capacity of insulin to regulate nutrient metabolism, and can initially be compensated by an increased secretion of insulin [1,2]. However, chronic secretion of large amounts of insulin to overcome tissue insensitivity can lead, in predisposed individuals, to pancreatic ß-cell failure and to the development of type II diabetes [3,4]. Understanding the origin of insulin resistance is of uttermost importance for the development of novel diagnostic and therapeutic tools to battle the expected burden of type II diabetes. Our recent data suggest that the endothelial glycocalyx may play a pivotal role in regulation of vascular insulin sensitivity and that damage to the endothelial glycocalyx may play a role in the pathogenesis of insulin resistance in obesity. First, we found that acute enzymatic degradation of the glycocalyx in rats resulted in an inability of insulin to recruit microvascular blood volume in muscle and this was accompanied by a decrease in insulin-mediated glucose disposal [5]. Next, we found evidence for glycocalyx degradation in muscle microcirculation in mice after already 6 weeks of a high fat diet (HFD). Of relevance, at this time point glucose intolerance seemed not fully manifested [6], indicating that glycocalyx damage may be an early event in diet-induced obesity (DIO) which may contribute to the development of glucose intolerance in this...
As a result, therapeutic interventions aiming at a recovery of the glyocalyx should be considered for improving insulin-mediated glucose regulation in DIO. In the current study we tested if the recently proposed glyocalyx-mimetic sulodexide would be able to alleviate insulin sensitivity in the HFD mouse model. Sulodexide is a glycosaminoglycan of natural origin extracted from mammalian intestinal mucosa, containing a mixture of 80% low-molecular-mass heparan sulphate and 20% dermatan sulphate [7]. Glycosaminoglycan chains contain numerous specific binding sites for plasma-derived proteins, and restoration of these chains may therefore improve functions of the glyocalyx [7]. As a reference treatment we used metformin, which is the most prescribed anti-hyperglycemic drug worldwide, and an anti-diabetic drug that has been shown to prevent cardiovascular complications [8]. It has been suggested that metformin improves glyocalyx dimensions as well, and this could be a possible explanation for the beneficial effects of metformin on endothelial function [9,10]. In the current study mice were fed a HFD for 6 or 18 weeks before which we performed an intraperitoneal glucose tolerance test (IPGTT) [6]. To evaluate the effect of the therapy, sulodexide and metformin were administrated via the drinking water for 2 weeks at the end of the feeding period.

**METHODS**

**Animals and diet**

All procedures were approved by the requirements of the Animal Ethics Care and Use committee of Maastricht University. After arrival from the external supplier (Harlan, Horst, The Netherlands) the animals (n=37) were housed at the animal facility of Maastricht University and received a HFD (Research Diets, New Brunswick, N), containing on caloric basis 60% fat, 20% carbohydrate and 20% protein for 6 or 18 weeks and water ad libitum. The HFD treated animals were divided into a group without therapy (6 weeks n=5, 18 weeks n=6), a group that received sulodexide (0.15 mg/mL; 6 weeks n=5, 18 weeks n=8), and a group that received metformin (0.3 mg/mL; 6 weeks n=5, 18 weeks n=8) in their drinking water during the last two weeks of the feeding period. Weekly (6 weeks HFD) or twice weekly (18 weeks HFD) measurements of body weight, blood pressure using a CODA non-invasive blood pressure monitoring system (Kent Scientific), and blood glucose levels and plasma insulin levels via blood sampling of the saphenous vein were performed in the conscious animal after a morning fast (4 h). Blood glucose (~ 5 μl blood) was measured with a glucose meter (Ascencia Contour). Further, about 40 μl blood was collected using a 75 μl glass capillary tube (Hirschmann, Germany) and centrifuged to collect plasma for the measurement of insulin levels with an ELISA (ALPCO Diagnostics, Salem, NH).

**Experimental protocol**

At the day of experiment, after an overnight fast (10-12 h), mice were anesthetized with an intraperitoneal injection (i.p.) of 0.39 mg/kg fentanyl, 7.81 mg/kg midazolam and 7.81 mg/kg acepromazine, and anesthesia was maintained by an additional bolus after 60-90 minutes of 0.10 mg/kg fentanyl, 1.56 mg/kg midazolam and 1.56 mg/kg acepromazine. The animal was put in a prone position and body temperature was maintained between 36°C and 37°C with the use of a heating pad and lamp and monitored with a rectal probe. During anesthesia a cannula was inserted intraperitoneally for glucose infusion [6].

**Intraperitoneal glucose tolerance test**

To determine glucose tolerance an IPGTT was performed. Mice were infused with a bolus of 1 g/kg glucose (0.1 gram/mL) via the i.p. cannula. Blood glucose was measured, via tail bleeding, with a glucose meter at t = -10 and 0 (pre) and t = 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes after the glucose infusion. About 40 μl blood was collected from the tail using a 75 μl glass capillary tube to determine systemic hematocrit and plasma insulin levels with an ELISA at t= 0 (pre) and t = 10, 30, 60 and 90 minutes after glucose infusion.

**Data analysis**

Effects of therapy on blood glucose and plasma insulin levels were evaluated during baseline and during the various time points of the tolerance test. Also, as a reflection of the circulating levels of glucose during the IPGTT we calculated the total area under the curve (AUC) of the glucose concentration versus time by the linear trapezoidal rule for the period of 0-120 minutes after glucose infusion (Figure 7.2A). Similarly, as a reflection of the circulating insulin levels we calculated the AUC of the insulin concentration versus time by the linear trapezoidal rule for the period of 0-90 minutes after glucose infusion (Figure 7.2B). As an index of baseline insulin resistance, the HOMA-IR ((Baseline glucose X baseline insulin)/22.5) was calculated [11], while as an index of peripheral insulin resistance during the IPGTT the IR-index, which is the product of the AUC of glucose and AUC of insulin was calculated [12].

All data are presented as means ± SEM. Statistical differences were tested with Student’s unpaired t-tests. A value of P<0.05 was considered statistically significant.

**RESULTS**

**Baseline characteristics**

Baseline characteristics of the HFD mice measured before the animals received the therapy, i.e., up to 16 weeks after start of the HFD, are shown in Figure 7.1. For reference, the characteristics in a group of mice on chow (presented in [6]) are shown as well (grey dashed line). As shown in our previous study [6], HFD was associated with increases in body weight, and baseline glucose and insulin levels, without a change in blood pressure. These parameters were not different between the three (no, sulodexide, or metformin treatment) groups. Neither metformin, nor sulodexide did have an effect on body weight in the mice that received a HFD for 6 weeks. However, compared to the body weight measured after feeding a HFD for 18 weeks without therapy (37.9 ± 1.6 grams), the body weight was lower after metformin treatment (33.2 ± 1.2 grams; P<0.05), while it tended to be decreased after sulodexide treatment (33.8 ± 1.4 grams; P=0.08).

**Ipgtt**

**Baseline:** Baseline glucose and insulin levels measured before the start of the IPGTT are shown in table 7.1. In the mice that were fed a HFD for 6 weeks baseline glucose and insulin
Figure 7.1 Mean ± SEM of body weight (A), blood pressure (B), and baseline glucose levels (C) and insulin levels in the 16 weeks that the mice received a HFD. The dashed grey line represents the values of the control group from our previous study [6]. After 16 weeks the mice received either sulodexide or metformin therapy in the final two weeks of the experiment, while the remaining groups did not receive any treatment.

Figure 7.2 In panel A the glucose levels during the IPGTT in the mice that received a HFD for 6 weeks without therapy, with sulodexide (SUL) or metformin (MET) therapy are shown while in panel B the corresponding AUC of glucose levels during the IPGTT are shown. The insulin levels during the IPGTT are shown in panel C and the AUC of insulin depicted in panel D. In panel E the IR-index (product of AUC glucose and AUC insulin) is shown. The dashed grey (panel A and C) or black line (panel B, D and E) represents the values of the control group (chow) from our previous study [6] *. P<0.05 compared to HFD.
Figure 7.3 In panel A the glucose levels during the IPGTT in the mice that received a HFD for 18 weeks without therapy or with sulodexide or metformin therapy are shown while in panel B the corresponding AUC of glucose levels during the IPGTT are shown. The insulin levels during the IPGTT are shown in panel C and the AUC of insulin are shown depicted in panel D. In panel E the IR-index (product AUC glucose and AUC insulin) is shown. The dashed grey (panel A and C) or black line (panel B, D and E) represents the values of the control group (chow) from our previous study [6]. * P<0.05 compared to HFD.

Figure 7.4 In panel A the relation between the AUC of glucose and AUC of insulin are shown for the mice that received a HFD for 6 weeks. In panel B the relation between the AUC of glucose and AUC of insulin are shown for the mice that received a HFD for 18 weeks. The data from the control group are the data obtained in [6].
levels were not affected by the two week sulodexide treatment. Baseline glucose levels were also not changed by metformin treatment, however baseline insulin levels were significantly increased (P<0.05) after metformin. In the mice that were fed a HFD for 18 weeks, baseline glucose and baseline insulin levels were not different in the mice that were treated by any of both therapies. The HOMA-IR was not affected by sulodexide or metformin after both a HFD for 6 weeks as well as for 18 weeks.

**Glucose tolerance test:** In the 6 weeks HFD fed mice, sulodexide treatment was associated with a significant decrease in blood glucose at t= 10, 20 and 30 minutes after glucose infusion (Figure 7.2, panel A). This decrease was, however, not sufficient to decrease the total AUC of the glucose levels significantly, yet this value was very close to the values obtained in our previous study in the chow group (Figure 7.2, panel B). Also, although insulin levels were not significantly decreased by sulodexide, the AUC of insulin was very close to that measured in the chow fed animals in our previous study. Metformin treatment did not improve glucose and insulin levels measured during the IPGTT in the 6 weeks HFD-fed mice.

In contrast to the effect of sulodexide on improvement of blood glucose levels during the IPGTT after 6 weeks of HFD, glucose levels were not significantly affected by this treatment in the mice that were fed the HFD for 18 weeks (Figure 7.3, panel A). Although there was also no significant difference in plasma insulin levels between the three groups during the IPGTT (Figure 7.3, panel C), the AUC of insulin was again close to the value observed in animals that received chow for 18 weeks in our previous study. The IR-index was significantly lower in the sulodexide treated mice compared to the non-treated mice.

**DISCUSSION**

Sulodexide is a glycosaminoglycan, containing a mixture of 80% low-molecular-mass heparan sulphate and 20% dermatan sulphate [7]. Given the abundance of these molecules in the endothelial glyocalyx, it may be considered to be a glyocalyx mimetic. Indeed, sulodexide treatment was recently shown to partially restore glyocalyx barrier properties in type II diabetic subjects [7], and this effect was associated with a reduction of the albumin clearance from the circulation in these subjects. It was not tested whether the improvement of the glyocalyx barrier properties by sulodexide was associated with an improvement in insulin sensitivity and glucose tolerance in the diabetic. In the current study we tested if short-term sulodexide would improve insulin-mediated glucose disposal at a relatively early and a later stage of diet-induced insulin resistance. Here for, mice were fed a HFD for 6 weeks or 18 weeks with or without sulodexide in their drinking water in the last 2 weeks of the feeding period. It was found that sulodexide improved blood glucose levels during an IPGTT in the face of similar insulin levels in the mice that were fed a HFD for 6 weeks. Furthermore, in the mice that were fed a HFD for 18 weeks, sulodexide improved the insulin resistance index. These data indicate that sulodexide improves insulin-mediated glucose regulation in DIO and suggest that recovery of the endothelial glyocalyx may be useful target in diabetes-prone conditions.

**RATIONALE OF THE STUDY**

The glyocalyx occupies a large volume of the microcirculation, and plays an important role in protection of the endothelium [13]. In addition to earlier studies in humans and experimental animals, in which it was shown that acute conditions of severe hyperglycemia [14,15] were associated with immediate glyocalyx damage, we recently provided evidence that glyocalyx damage also developed in the early stages of diet-induced obesity, before the presence of overt glucose intolerance. Together with the finding that acute enzymatic degradation of the glyocalyx resulted in an impaired insulin-mediated glucose disposal [5], these data suggest that glyocalyx damage may not only be a consequence of a defective insulin action and resultant hyperglycemia but may actually contribute to them. As a result, the glyocalyx may be a worthwhile target for therapy directed at improvement of insulin sensitivity in DIO. Since previous studies in rodents had shown that infusion of glyocalyx components, such as hyaluronan [16] and heparan sulfates [17], could restore a degraded glyocalyx and its protective function, we hypothesized in the current study that sulodexide would improve insulin sensitivity in diet-induced obese mice.

**METHODOLOGICAL CONSIDERATIONS**

**HFD**

The HFD-fed mouse model has been used to study mechanisms of impaired glucose tolerance and type II diabetes [18,19] and to evaluate new treatments [20,21]. In our previous study we showed that feeding a HFD for 6 weeks in mice did not affect baseline glucose and insulin levels and was associated with rather mild increases in glucose and compensatory insulin secretion during the IPGTT, which did not yet result in a significant increase in the total AUC of glucose or insulin levels during the test [6]. In the current study, we also calculated the IR-index as the product of the AUC of both parameters [22,23]. It has been argued by Matsuda and DeFronzo that this index provides a better reflection of muscle insulin resistance during a glucose tolerance test than for example the ratio of both parameters, since normal or higher glucose levels with higher insulin levels reflects the presence of insulin resistance [12]. The IR-index (Figure 7.2E) was not significantly increased in the mice that were fed a HFD for 6 weeks, indicating that insulin resistance was not fully present at this stage (Figure 7.4, left panel). After 18 weeks of HFD baseline insulin and glucose levels were also not significantly changed but glucose disposal appeared impaired for the entire 2 h after the glucose infusion, resulting in a significantly elevated AUC of glucose. In addition, insulin levels tended to be increased as well. These data indicate that after feeding a HFD for 18 weeks, insulin resistance was more aggravated and indeed the IR-index was clearly increased at this stage (Figure 7.3E).

**Treatments**

Sulodexide was used in previous clinical studies to test the effect in diabetic nephropathy, and it has been shown that sulodexide treatment for several weeks was associated with an improvement in albuminuria in type I and II diabetic patients [24,25]. However, other studies showed that sulodexide did not have a renoprotective benefit and was ineffective in decreasing urine albumin excretion in type 2 diabetic patients. As a result,
Table 7.1: Baseline circulating glucose and insulin values (measured before the start of the IPGTT).

<table>
<thead>
<tr>
<th>Therapy</th>
<th>HFD for 6 weeks</th>
<th>HFD for 18 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without</td>
<td>Sulodexide</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>6.8 ± 0.6</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Plasma Insulin (µU/ml)</td>
<td>9.7 ± 0.4</td>
<td>11.0 ± 1.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.81 ± 0.32</td>
<td>3.11 ± 0.58</td>
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Baseline glucose and insulin levels measured during anesthesia before the start of the IPGTT in mice that were fed a HFD for 6 or 18 weeks, respectively. As a measurement for baseline insulin resistance the HOMA-IR ((Baseline glucose X baseline insulin)/22.5) was calculated. *, P<0.05 compared to HFD mice without treatment.

The results of the current study show that short-term treatment with sulodexide, a presumed glycocalyx mimetic, improves insulin sensitivity in diet-induced obese mice. In addition to the previous reported beneficial effects of sulodexide on glycocalyx barrier properties, this suggests that sulodexide may hinder the development of glucose intolerance and ultimately type II diabetes. Sulodexide could, therefore, be a potential new therapeutic for obese people predisposed for developing insulin resistance.

REFERENCES


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