A Fermented Soy Permeate Improves the Skeletal Muscle Glucose Level Without Restoring the Glycogen Content in Streptozotocin-Induced Diabetic Rats

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ABSTRACT Exercise is essential into the therapeutic management of diabetic patients, but their level of exercise tolerance is lowered due to alterations of glucose metabolism. As soy isoflavones have been shown to improve glucose metabolism, this study aimed to assess the effects of a dietary supplement containing soy isoflavones and alpha-galactooligosaccharides on muscular glucose, glycogen synthase (GSase), and glycogen content in a type 1 diabetic animal model. The dietary supplement tested was a patented compound, Fermented Soy Permeate (FSP), developed by the French Company Sojasun Technologies. Forty male Wistar rats were randomly assigned to control or diabetic groups (streptozotocin, 45 mg/kg). Each group was then divided into placebo or FSP-supplemented groups. Both groups received by oral gavage, respectively, water or diluted FSP (0.1 g/day), daily for a period of 3 weeks. At the end of the protocol, glycemia was noticed after a 24-h fasting period. Glucose, total GSase, and the glycogen content were determined in the skeletal muscle (gastrocnemius). Diabetic animals showed a higher blood glucose concentration, but a lower glucose and glycogen muscle content than controls. Three weeks of FSP consumption allowed to restore the muscle glucose concentration, but failed to reduce glycemia and to normalize the glycogen content in diabetic rats. Furthermore, the glycogen content was increased in FSP-supplemented controls compared to placebo controls. Our results demonstrated that diabetic rats exhibited a depleted muscle glycogen content (–25%). FSP-supplementation normalized the muscle glucose level without restoring the glycogen content in diabetic rats. However, it succeeded to increase it in the control group (+20%).

KEY WORDS: • *alpha-galactooligosaccharides* • *glucose metabolism* • *soy isoflavones* • *type 1 diabetes*

PHYSICAL ACTIVITY IS now well integrated into the therapeutic management of diabetic patients to improve glycemic control and quality of life.¹ Nevertheless, the level of exercise tolerance in patients with insulin-dependent diabetes mellitus (IDDM) is lower than the level of exercise tolerance of healthy people.² One explanation could be the decreased glycogen content in the skeletal muscle of IDDM patients,^{3,4} but literature data remain controversial.

Soy isoflavones have beneficial effects in IDDM management:⁵ the consumption of genistein or daidzein reduces the blood glucose level and improves insulinemia in type 1 diabetic rats.^{6–8} To our knowledge, no study has investigated the effects of soy isoflavones on glycogen synthesis in the muscle of a type 1 diabetic animal model. A French company, Sojasun Technologies, has developed a powder ex-

tracted from the soybean called Fermented Soy Permeate (FSP). The specificity of this product is due to the presence of two types of active molecules from the soybean: isoflavones and alpha-galactooligosaccharides (alpha-GOS), which potentiate the absorption of isoflavones.⁹ The advantage is to keep the natural association of soy active compounds, which provide synergistic effects.

We aimed to assess that:

- the intramuscular glycogen content is indeed lower in a type 1 diabetic rat model,
- FSP could prevent this glycogen storage deficiency by improving several steps of glycogen synthesis in diabetics.

The experimental protocol, approved by the Ethics Committee of Rennes University, was in accordance with the Guide for the Care and Use of the Laboratory Animals. Forty 9-week-old male Wistar rats (Janvier) were randomly assigned to the control or diabetic groups. Then, each group was divided into placebo or FSP-supplemented groups. Tap water and food (2020X Teklad Global Soy Protein-Free; Harlan) were available *ad libitum*. Food intake was daily recorded.

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TABLE 1. EFFECTS OF DIABETES AND FERMENTED SOY PERMEATE SUPPLEMENTATION ON GLYCEMIC CONTROL, GLUCOSE METABOLISM IN SKELETAL MUSCLE, AND FOOD INTAKE

	Controls		Diabetics	
	Placebo (n = 10)	FSP (n = 10)	Placebo (n = 10)	FSP (n = 10)
Blood glucose level (mmol/L)	5.26 ± 0.12	4.90 ± 0.46	19.99 ± 1.76**	22.37 ± 0.89
Serum fructosamine level (μmol/L)	127.38 ± 4.05	138.29 ± 4.89	209.60 ± 20.18**	239.71 ± 12.31
Muscle glucose concentration (μmol/100 mg tissue)	0.75 ± 0.09	0.73 ± 0.08	0.59 ± 0.14*	0.78 ± 0.12***
Total muscle glycogen synthase content (mg/100 mg tissue)	11.55 ± 4.25	9.31 ± 0.07	7.26 ± 3.15	8.04 ± 5.10
Muscle glycogen content (μmol glucosyl equivalents/100 mg tissue)	2.51 ± 0.23	2.99 ± 0.58***	1.89 ± 0.27**	2.05 ± 0.56
Food intake (g/day)	28.84 ± 1.26	29.50 ± 2.01	59.25 ± 6.93**	58.60 ± 4.17

Values are means ± SEM, after 3 weeks of daily gavage with FSP or placebo (water), in control or streptozotocin-induced diabetic rats.

Effect of diabetes: significant difference between placebo diabetic animals versus placebo controls (* $P < .05$; ** $P < .01$).

Effect of supplementation: significant difference between FSP-supplemented versus placebo (*** $P < .05$), in diabetic or control groups.

FSP, Fermented Soy Permeate.

Diabetes was induced by intraperitoneal streptozotocin injection (45 mg/kg of body weight, citrate buffer). Controls received the citrate buffer alone. The blood glucose concentration was measured 48 h later (MediSense Optium® glucometer; Abbott); animals with blood glucose levels greater than 250 mg/dL were considered diabetic.¹⁰

The dietary supplement tested is a patented compound,¹¹ FSP, developed by Sojasun Technologies. The product was a soluble powder containing 29% alpha-GOS and 0.5% soy isoflavones (daidzein and genistein).

One week after the confirmation of diabetes, animals started to receive by oral gavage FSP-supplementation or water (daily for a period of 3 weeks). Volume of FSP or water was daily adjusted according to body weight, to ensure that each rat received a FSP dose equivalent to 1 mg of soy isoflavones and 70 mg of alpha-GOS/kg of body weight each day.

Then, all rats were sacrificed after a 24 h-fasting period, and the blood glucose and fructosamine levels were measured (Cerbera). The other parameters were determined in gastrocnemius. The glucose concentration was determined using a Glucose GOD-PAP Assay kit (Biolabo). The glycogen content was measured with the same assay kit after total hydrolysis of the glycogen into glucose using alpha-amylase. The total glycogen synthase (GSase) content was determined using a GSase (Total) Elisa kit (Invitrogen). All results were normalized by muscle weight to account for individual variations in the animal's body weights during the experiment.

The results are expressed as the means ± SEM. The effect of diabetes was assessed using a Mann-Whitney test between the two placebo groups. The effects of FSP supplementation on both groups were determined using analysis of variance (Student-Newman-Keuls *post hoc* test). The Spearman test was used to detect correlations.

As expected, the final blood glucose and fructosamine levels were higher in diabetic rats than in controls. In muscle, diabetic rats exhibited lower glucose concentrations

and glycogen contents, although the GSase content was not affected by diabetes (Table 1). FSP supplementation had no effect on the blood glucose and fructosamine levels, but restored the glucose concentration in the muscle of diabetic animals. Moreover, FSP consumption increased the muscle glycogen content in supplemented control rats compared with the placebo control rats. No change in the GSase content was observed after 3 weeks of FSP supplementation in either the diabetic or placebo groups. No correlation was found among all parameters for the four groups.

The major results of this study were that (1) diabetes leads to a lower muscle glycogen content, and (2) FSP supplementation restored the muscle glucose content in diabetic rats, but not the depleted glycogen store. However, FSP supplementation increased the glycogen store in controls (+20%).

The diabetic state was confirmed by the high blood glucose and fructosamine levels at the end of the protocol. Despite the high blood glucose concentration, diabetic rats exhibited lower muscle glucose levels than controls, indicating that glucose transport into muscle cells was disrupted in the diabetic rats.³ Given that insulin is the major factor regulating the muscle glucose uptake, this disruption may result from insulin deficiency in diabetic rats, as previously reported in the same animal model.¹²

As hypothesized, in placebo diabetic rats, the lower muscle glucose concentration led to lower muscle glycogen content. Literature data concerning the muscle glycogen content are often inconsistent, with a decrease,¹³ no change,⁴ and even an increase¹⁴ reported in diabetics relative to healthy subjects. These conflicting results could be explained by (1) the different methods to determine the glycogen content (possibly leading to incomplete glycogen degradation before quantification) and (2) the different nutritional states of the rats (fed or fasted). Indeed, the glycogen storage depends on the food consumption,¹⁵ which was twofold higher in diabetics than in controls. In this experiment, the analysis of fasted rats ensured that all

animals were in similar conditions and that the lower glycogen content resulted from the diabetic state and not from the rats being in different postprandial states.

Glycogen is synthesized from glucose through a succession of enzymatic reactions. Because no correlation was found between the muscle glucose level and the muscle glycogen content, the intermediate steps could be disrupted. For example, glucose phosphorylation could be defective, and GSase regulation could be disrupted.³ As it has been demonstrated that the GSase phosphorylation ratio was not affected by the diabetic state,¹⁴ we evaluated the total GSase content, which was not affected by the diabetic state nor upregulated to counteract the lower glycogen content.

In our study, FSP consumption was unable to improve glycemic control (blood glucose and fructosamine levels) in diabetic rats. To our knowledge, the literature data showing positive effects of isoflavones on the blood glucose level^{6–8} used supra-physiological doses (equivalent to 25 to 75 mg/kg body weight vs. 2 mg/kg) and treated the animals over a longer period (up to 8 weeks vs. 3 weeks). Our study aimed to determine the effects of FSP at physiological doses; thus, the administration of a lower soy isoflavones dose could explain the lack of improvement in the blood glucose level.

Nevertheless, physiological FSP supplementation was able to restore the muscle glucose content in diabetic rats. This beneficial effect could be explained by the elevation of the insulin level induced by soy isoflavones, an effect that is well described in type 1 diabetic animal models⁶ and in streptozotocin-induced diabetic rats in particular.^{7,8} Indeed, Lu *et al.*⁷ observed a significant restoration of the structure of Langerhans' islets and of insulin production in diabetic rats. In addition, Liu *et al.* reported an insulinotropic effect of genistein at physiological doses on pancreatic β -cells.¹⁶

Despite the restoration of the muscle glucose concentration in the diabetic supplemented group, the muscle glycogen content remained low. In contrast, the consumption of FSP in the control group induced an increase in the glycogen content. These opposite results could be explained by the regulation of glycogen synthesis in muscle. This synthesis depends on the glycogen content: in case of glycogen depletion in muscle, glycogen synthesis is activated by hyperglycemia, whereas at basal levels, glycogen synthesis is activated by insulin.¹⁷ In diabetic subjects, because the glycogen stores are chronically depleted, blood glucose becomes the primary stimulus that activates glycogen synthesis, which allows the preservation of partial glycogen synthesis despite lower insulinemia,¹⁸ but FSP had no effect on this phenomenon. In the control group, the increased glycogen content resulted from the stimulatory effect of FSP on insulin secretion, as previously explained.

In summary, FSP supplementation may increase the glucose transport from the blood into the muscle in controls and diabetics groups. In controls, FSP supplementation stimulated the conversion of intramuscular glucose into glycogen, leading to an increased glycogen storage. In diabetics, FSP supplementation had no effect on the different steps of glycogen synthesis, leading to an accumulation of glucose in the muscle.

To conclude, type 1 diabetes induced a decrease in the glucose and glycogen levels in muscle. Three weeks of consumption of FSP, a dietary supplement containing soy isoflavones, (1) partially corrected diabetes-induced changes, allowing the restoration of the muscle glucose content in the diabetic group, and (2) increased the glycogen content in the muscles of the control group. This short communication highlights the interest in FSP development and is the first step in future research investigating underlying mechanism and FSP's action in various areas.

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AUTHOR DISCLOSURE STATEMENT

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