ISBN 978-85-61091-05-7

VI EPCC
Encontro Internacional de Produção Científica Cesumar
27 a 30 de outubro de 2009

THE EFFECTS OF FISETIN ON GLUCONEOGENESIS IN THE RAT LIVER

Rodrigo Polimeni Constantin[1]; Renato Polimeni Constantin; Cristiane Vizioli de Castro; Nair Seiko Yamamoto[3]

ABSTRACT: Flavonoids have been proposed to exert beneficial effects in a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders. The most known biological effects of the flavonoids, is no doubt their antioxidant action, which represents protection of tissues against the action of free-radicals and diminution of lipid peroxidation. Fisetin is a flavonoid found in the smoke tree (*Cotinus coggyria*) and is also widely distributed in fruits and vegetables such as strawberry, apple, persimmon, grape, onion, and cucumber. It has been reported to suppress the proliferation of tumor cells, such as prostate cancer, liver cancer, colon cancer, and leukemia. The present work was planned to investigate the effects of fisetin on gluconeogenesis in the rat liver. Livers from 24 h fasted Male Wistar rats, weighing 200 to 280 g rats, were used for the measurement of gluconeogenesis. The isolated liver was perfused in the non-recirculating system. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with a mixture of O_2 and CO_2 (95:5). Gluconeogenesis from lactate and pyruvate in the liver from fasted rats was inhibited in a concentration-dependent manner. Transformation of fructose into glucose and the oxygen uptake increase accompanying gluconeogenesis were also inhibited by fisetin. The data of this work suggest that fisetin inhibits glucose release from the liver and could be potentially useful as an antidiabetic agent, although further studies are needed to validate its therapeutic use.

KEY WORDS: Fisetin; Flavonoids; Gluconeogenesis; Liver.

1 INTRODUCTION

Fisetin (3,7,3´,4´-tetrahydroxiflavone) is a flavonoid dietary ingredient found in the smoke tree (*Cotinus coggyria*) and is also found in fruits and vegetables such as strawberry, apple, persimmon, grape, onion, and cucumber at concentrations of 2 to 160 µg/g (ARAI et al., 2000). It exhibits a wide variety of pharmacological properties, including neurotrophic, antioxidant, anti-inflammatory and antioangiogenic effects. It has been reported to suppress the proliferation of tumor cells, such as prostate cancer, liver cancer, colon cancer, and leukemia. Certain bioflavonoids, including fisetin, inhibit glycolysis in Ehrlich ascites tumor cells (SUOLINNA et al., 1974; SUOLINNA et al., 1975).

Flavonoids have also been identified as the antidiabetic components in a number of traditional ethnic remedies (JUNG et al., 2006). Although there has been considerable scientific progress over the past few years in the unraveling of the effect and mechanism of action of flavonoids, the mechanisms whereby these compounds exert their

[1] Doutorando em Ciências Biológicas. Área de Concentração em Biologia Celular e Molecular. Departamento de Bioquímica, Laboratório de Metabolismo Hepático, Universidade Estadual de Maringá (UEM), Maringá – PR. rrconstantin@hotmail.com
[2] Acadêmicos do curso de farmácia. Departamento de Bioquímica, Laboratório de Metabolismo Hepático, Universidade Estadual de Maringá (UEM), Maringá – PR. Bolsistas do Programa de Bolsas de Iniciação Científica do PIBIC/CNPq-UEM (PIBIC-UEM). rereconstantin@yahoo.com, crisvizioli@gmail.com
[3] Docente da Universidade Estadual de Maringá. Departamento de Bioquímica, Laboratório de Metabolismo Hepático, Universidade Estadual de Maringá (UEM), Maringá – PR. nsyamamoto@uem.br

hypoglycemic action have rarely been investigated. There are indications that the liver is implicated in this hypoglycemic action.

In groups of mice whose diet was supplemented with the flavonoids hesperedin and naringin, increased glucokinase activity and the glycogen content was found bigger than before. Naringin also reduced the activity of hepatic glucose 6-phosphatase and phosphoenolpyruvate carboxykinase (JUNG et al., 2004). The authors have suggested that hesperedin and naringin improve hyperglycemia by regulating the activity of these hepatic enzymes involved in glycolysis and gluconeogenesis (JUNG et al., 2004).

There is no information about the actions of the flavonol fisetin on liver glucose metabolism. Thus, we here report the study of the potential acute effects of fisetin on gluconeogenesis in the isolated perfused rat liver.

2 MATERIALS AND METHODS

The liver perfusion apparatus was built in the workshops of the University of Maringá. Fisetin was purchased from Sigma-Aldrich (ST Louis, MO, USA). Enzymes and coenzymes used in the enzymatic assays were purchased from Sigma-Aldrich (St Louis, MO, USA). Male Wistar rats (weighing 200-280 g) fed with a standard laboratory diet (Nuvital - Nuvilab CR-1®) were used in all experiments. All experiments were conducted in strict adherence to the guidelines of the Ethics Committee for Animal Experiments of the University of Maringá. For the surgical procedure, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg^{-1}). Haemoglobin-free, non-recirculating perfusion was undertaken. After cannulation of the portal and cava veins, the liver was positioned in a plexiglas chamber. Flow was maintained constant by a peristaltic pump (Miniplus 3, Gilson, France) and was adjusted to between 30 and 35 ml min^{-1}, depending on the liver weight.

The perfusion fluid was Krebs/Henseleit bicarbonate buffer (pH 7.4), saturated with a mixture of oxygen and carbon dioxide (95:5) by means of a membrane oxygenator with simultaneous temperature adjustment at 37°C. The composition of the Krebs/Henseleit bicarbonate buffer is as follows: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM $MgCl_2$, 1.2 mM NaH_2PO_4 and 2.5 mM $CaCl_2$. Fisetin was dissolved in the perfusion fluid. Solubilization was achieved by the simultaneous addition of an equivalent amount of NaOH. Livers from 24 h fasted rats were used for the measurement of gluconeogenesis.

Samples of the effluent perfusion fluid were collected at 2-min intervals and analyzed for their metabolite content. Lactate and pyruvate were assayed by means of standard enzymatic procedures using L-lactate dehydrogenase. Interference by fisetin (absorbance at 340 nm) was excluded by running blanks. The oxygen concentration in the outflowing perfusate was monitored polarographically, employing a Teflon-shielded platinum electrode adequately positioned in a plexiglas chamber at the exit of the perfusate (CLARK, 1956). Fisetin interferes with the glucose oxidase reaction and, for this reason, glucose was measured colorimetrically by means of an *o*-toluidine method (DUBOWSKI, 1962).

The statistical significance of the differences between parameters was evaluated by means of Student's t test or Newman-Keuls test. The latter was applied after submitting the data to variance analysis. The results are mentioned in the text as the p values; $p<0.05$ was adopted as a criterion of significance.

3 RESULTS AND DISCUSSION

The action of fisetin on gluconeogenesis from lactate and pyruvate was evaluated according to the protocol illustrated by figure 1 in perfused livers from 24-h fasted rats.

Under this condition, during the time period before lactate (2.0 mM) plus pyruvate (0.2 mM) infusion, glucose release was minimal due to the low endogenous levels of glycogen and gluconeogenic substrates. Both gluconeogenesis and oxygen consumption increased upon addition of the exogenously supplied gluconeogenic substrates, tending to stabilize at 20 min infusion time. The introduction of 300 µM fisetin produced progressive decreases in both gluconeogenesis and oxygen consumption. At the end of the infusion, glucose production was reduced by 72% (p<0.001) when compared with the rates measured before the infusion of the drug. These effects were partially reversible, that is, when the infusion of the drug was interrupted at 50 minutes, the metabolic fluxes tended to return slowly to the rates before its infusion.

Figure 2 allows an evaluation of the changes caused by several concentrations of fisetin in the range of 50 to 300 µM on oxygen uptake and gluconeogenesis. Gluconeogenesis was reduced in a dose-dependent manner. Inhibition of gluconeogenesis was already evident at the concentration of 50 µM and increased considerably when the concentration of the drug was raised to 300 µM (72%, p<0.001).

Fructose can also be converted into glucose, but ramification of the fructose pathway at the enolase step leads also to the production of lactate and pyruvate. The action of fisetin on fructose metabolism was investigated in experiments like those shown in Figure 1. The results summarized in figure 3 show that glucose production from exogenously supplied fructose was inhibited 43% (p<0.01) by 300 µM fisetin. Lactate and pyruvate production were also reduced; the inhibition reached 72% (p<0.01) and 60% (p<0.01) respectively, while oxygen consumption decreased 16% (p<0.01) in the presence of fisetin.

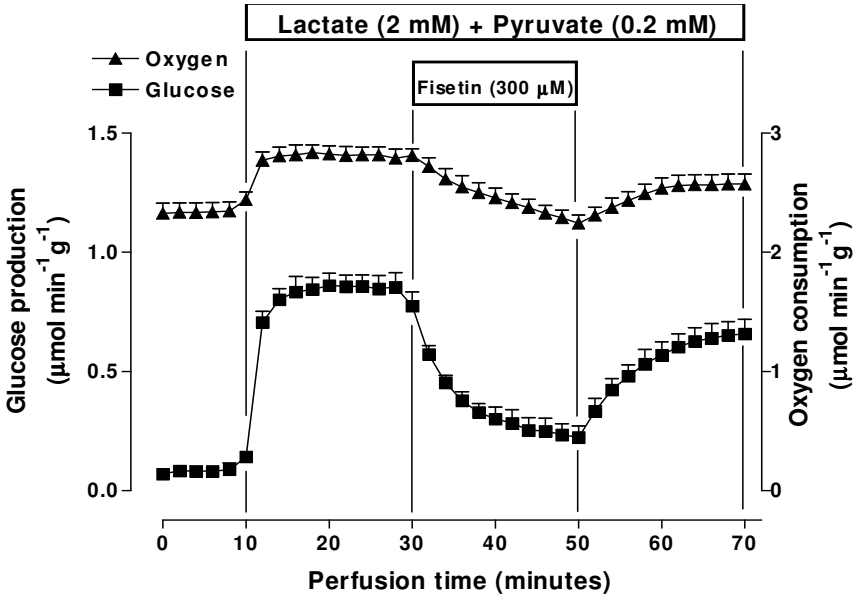

Figure 1. Effects of fisetin on metabolic fluxes in perfused livers isolated from fasted rats. Time course of the changes caused by fisetin 300 µM in glucose production and oxygen consumption. Livers from fasted rats were perfused as described in Materials and methods. Lactate (2 mM) and pyruvate (0.2 mM) were infused at 10-70 min and fisetin at 30-50 min as indicated by the horizontal bars.

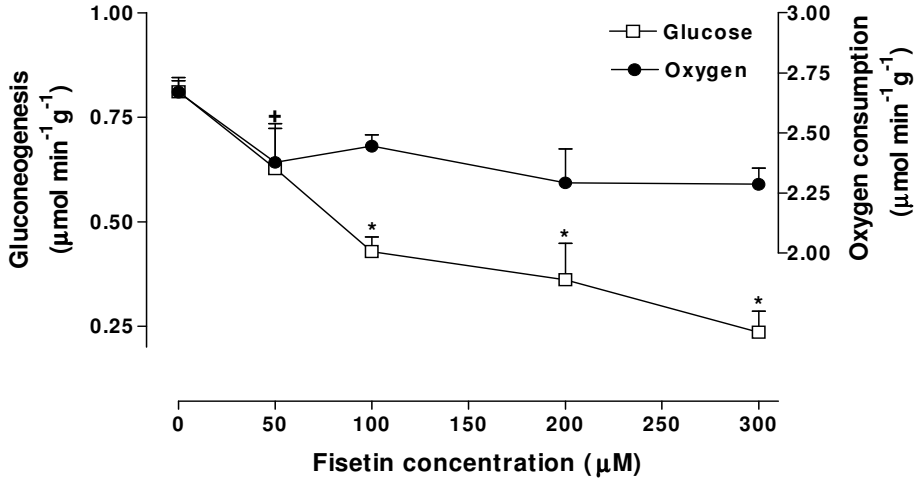

Figure 2. Effects of fisetin on metabolic fluxes in perfused livers isolated from fasted rats. Concentration dependence of the effects of fisetin on oxygen consumption and gluconeogenesis. The experimental protocol was the same described for figure 2. Each data point is the mean ± SEM of four experiments. Asterisks and crosses indicate statistical significance in comparison with the control condition as revealed by variance analysis with post hoc Newman-Keuls testing ($^{+}p<0.05$, $^{*}p<0.001$).

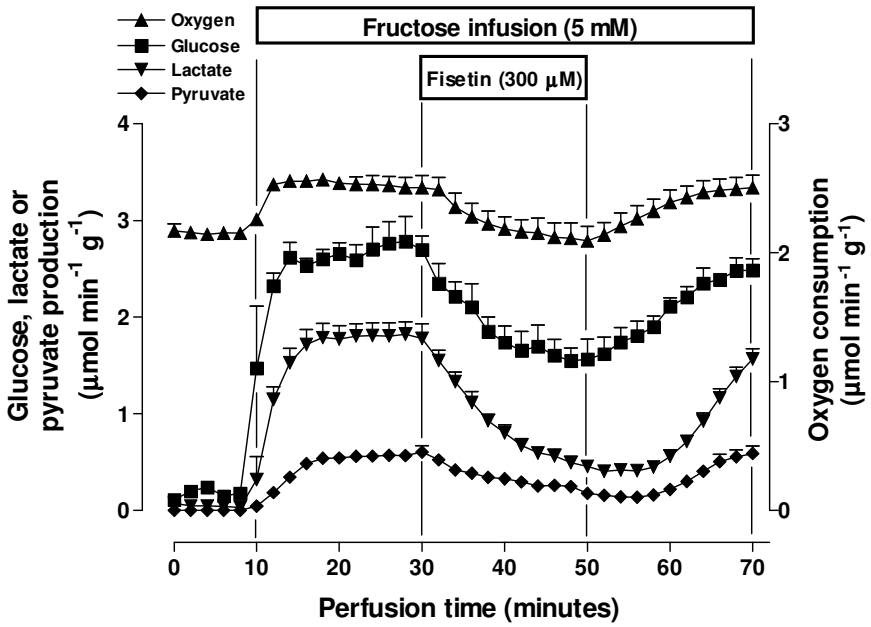

Figure 3. Effects of fisetin on fructose metabolism. Livers from fasted rats were perfused with Krebs/Henseleit-bicarbonate buffer (pH 7.4), as described in Materials and methods. Fructose (5 mM) was infused at 10-70 min and fisetin (300 µM) at 30-50 min as indicated by the horizontal bars. The effluent perfusate was sampled in 2-min intervals and analyzed for glucose, lactate and pyruvate. Oxygen consumption was followed polarographically. Each experimental point is the mean ± SEM of three experiments with identical protocol.

4 CONCLUSION

The data of this work suggest that fisetin inhibits glucose production from lactate, pyruvate and fructose in perfused livers. It is concluded that fisetin could prevent hyperglycemia by lowering hepatic gluconeogenesis. In fact, it is considered that dietary bioflavonoids may offer some protection against the early stage of diabetes mellitus (JUNG et al., 2004), but further studies are needed to validate its therapeutic use.

REFERENCES

ARAI, Y.; WATANABE, S.; KIMIRA, M.; SHIMOI, K.; MOCHIZUKI, R.; KINAE, N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. **Journal of Nutrition**, v. 130, n. 9, p. 2243-2250, 2000.

CLARK, L.M. Monitoring and control of blood O_2 tension. **Transactions-American Society for Artificial Internal Organs** v. 2, p. 41-49, 1956.

DUBOWSKI, K.M. An *o*-toluidine method for body-fluid glucose determination. **Clinical Chemistry**, v. 8, n. 3, p. 215-235, 1962.

JUNG, U.J.; LEE, M.K.; JEONG, K.S.; CHOI, M.S. The hypoglycemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. **The Journal of Nutrition**, v. 134, n. 10, p. 2499-2503, 2004.

JUNG, U.J.; LEE, M.K.; PARK, Y.B.; KANG, M.A.; CHOI, M.S. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. **The International Journal of Biochemistry & Cell Biology**, v. 38, n. 7, p. 1134-1145, 2006.

SUOLINNA, E.M.; BUCHSBAUM, D.N.; RACKER, E. The effect of flavonoids on aerobic glycolysis and growth of tumor cells. **Cancer Research** v. 35, n. 7, p. 1865-1872, 1975.

SUOLINNA, E.M.; LANG, D.; RACKER, E. Quercetin, an artificial regulator of the high aerobic glycolysis of tumor cells. **Journal of the National Cancer Institute** v. 53, n. 5, p. 1515-1519, 1974.