Effects of Vanadate on Glucose Production in Cultured Hepatocytes Isolated from Rats on High Saturated Fat Diet

Roša, Jagoda; Skala, Hana; Roša, Josip

Source / Izvornik: **Collegium antropologicum, 2005, 29, 693 - 696**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:220:460983

Coll. Antropol. **29** (2005) 2: 693–696
UDC 576.34:616.379-008.64
Original scientific paper

Effects of Vanadate on Glucose Production in Cultured Hepatocytes Isolated from Rats on High Saturated Fat Diet

Jagoda Roša[1], Hana Skala[2] and Josip Roša[2]

[1] Department of Physiology, School of Dental Medicine, University of Zagreb, Zagreb, Croatia
[2] Department of Physiology, School of Medicine, University of Zagreb, Zagreb, Croatia

A B S T R A C T

Insulin resistance is a common phenomenon in obesity and Type 2 diabetes. Common factor important for development of diabetes and insulin resistance is intake of saturated fat. Vanadate treatment improves glucose homeostasis in vivo. The aim of this study was to find out changing of hepatic glucose output in dependence of saturated fat diet and possible direct action of vanadate in cultured hepatocytes. Hepatocytes were isolated by a collagenase perfusion technique and cultured for 24 h in M 199 serum-free medium. The glucose production in hepatocytes isolated from rats on high saturated fat diet was significantly 139% higher comparable to standard controls. Glucagon 100% increased glucose production in hepatocytes from rats on standard diet and 200% in hepatocytes on saturated high fat diet. The addition vanadate significantly decreased basic glucose production and did not influence glucagon stimulated glucose production. Presence of insulin did not influence either glucagon or vanadate effect. High saturated fat diet not only increases insulin resistance but also decreases chances of successful therapy of diabetes.

***Key words**: insulin, glucagon, glucose production, gluconeogenesis, vanadate*

Introduction

A high level of dietary fat intake in the Western diet is believed to be a major factor in the development of insulin resistance[1,2]. Insulin resistance is a common phenomenon in obesity and type 2 diabetes[3]. Critical insights into the etiology of insulin resistance have been gained by the use of animal models where insulin action has been modulated by strictly controlled dietary interventions. Intake of saturated fats is strongly linked to development of obesity and insulin resistance. This is consistent with observation that saturated fats are poorly oxidized for energy and thus readily stored[4]. Although studies in certain population subgroups show a positive correlation between amount of dietary fat intake and occurrence of type 2 diabetes, these cannot be considered definitive, in part because they are confounded by many other variables that might relate to the development of diabetes. With regard to diabetes risk, type of dietary fat consumed may be more important than total dietary fat intake[5]. So common factor important for development of diabetes and insulin resistance is intake of saturated fats. Rats on high saturated fat diet had a greater hepatic glucose production comparable with rats on standard diet[6]. Increased hepatic glucose production correlates well with fasting glucose levels and is the main cause of fasting hyperglycemia in type 2 diabetes[7]. Insulin resistance is a prevalent condition in which insulin loses its normal physiological action. Small oral doses of vanadyl sulfate improve both hepatic and the skeletal insulin sensitivity. In vivo vanadate increases insulin mediated glucose uptake in human type 2 diabetes[8]. We showed that vanadate inhibited gluconeogenesis in liver[9]. This is especially important in conditions where process of gluconeogenesis is very active, like in diabetic animals or animals held on high fat diet. Vanadate indeed completely normalized basal glucose production and 50% decreased glucagon stimulated glucose production in cultured hepatocytes isolated from rats held on high unsaturated fat diet[10]. Since saturated fats significantly increased insulin resistance, long and short-chain omega-3 fatty acids significantly improved it, whereas the effect of unsaturated fatty acids ranged somewhere in between, we

Received for publication February 11, 2005

changed macronutrient proportion of fat diet from high unsaturated to high saturated fat, using animal fat. In this study we tried to find out changing of hepatic glucose output in dependence of saturated fat diet and possible direct action of vanadate in cultured hepatocytes.

Material and Methods

In all experiments, male adult Wistar rats, each weighting 250–325 g. was used. Rats were housed individually in wire cages in a temperature-controlled room (21±1°C), on 12 h light-dark cycle, with free access to food and water for three weeks. High fat diet we prepare mixing 30% of animal fat to standard food. High fat diet contained 30% carbohydrates, 16% protein and 54% fat, as we already reported[6].

Principles of animal care (NIH publication No. 85–23, revised 1985) were followed.

Hepatocytes were isolated by a modified collagenase-perfusion technique[11]. The rats were anaesthetized with Phenobarbital (10 mg/100 g body weight) and calcium-free Swim's S-77 medium containing collagenase (0.5 g/l) was used for liver perfusion through a portal cannula. Usually more than 90% of cells excluded trypan blue as the measure of viability. After washing twice with the same collagenase-free medium, the cells were suspended to a final concentration of a one million cells per ml M199 serum-free medium. Three ml of cell suspension was placed in 60 mm Petri dishes previously coated with collagen. Culture dishes were kept at 37°C in an atmosphere of 5% CO_2 and 95% air (CO_2 incubator Heraeus, Hanau, Germany). The culture medium was replaced with fresh medium 4 hours later to remove unattached cells and hepatocytes were incubated for the next 24 hours in the M199 serum-free medium.

After having been 24 h in culture, the medium was removed and cells were incubated in glucose-free Hanks--Hepes medium, containing 10 mmol/l pyruvate, without hormones (control) or insulin (80 nmol/l), glucagon (0,2 μmol/l) or vanadate (1 mmol/l). The glucose released into the medium was determined enzymatically with glucose oxidase. The glucose production was measured by incubating the cultures in glucose-free Hanks- Hepes medium with addition 10 mmol/l pyruvate. The incubation medium was removed and hepatocytes were washed three times with cold saline and frozen immediately in liquid nitrogen. The cells were digested in 0.2 N NaOH and an aliquot was taken for the determination of protein.

Albumin bovine, glutamine, HEPES, M199 medium, Swim's S-77 medium, insulin, vanadate were obtained from Sigma; Collagenase CLS II (131 U/mg) was purchased from Worthington; Collagen R was purchased from Serva.

Perfusion medium is a Swim's S-77 medium containing 2.2 g $NaHCO_3$ and 585 mg glutamine per litter.

Incubation medium is a M199 medium containing the following additions per litter: 2 g albumen, 900 mg L-glutamine and 2.2 g $NaHCO_3$.

Data are expressed as means ± SEM. Statistical significance was evaluated by Student's t-test. $P<0.05$ was considered statistically significant.

Results

In cultured hepatocytes isolated from rats on high saturated fat diet, there was an almost linear increase of glucose production throughout the all 3-hours period and in first hour was 90% higher, in second hour 96% and in third hour 139% higher than in control cultures. The addition of vanadate (1 mmol/l) significantly decreased glucose production in whole 3-hours period and after three hours it was 21% lower than in untreated controls. Insulin alone, in a concentration of 80 nmol/l, also significantly decreased the glucose production in hepatocytes isolated from rats on high fat diet and after three hours it was 15% lower than in untreated controls. Insulin is less effective than vanadate and more interestingly effect almost despaired during simultaneous insulin and vanadate treatment (Figure 1).

Glucagon significantly increased glucose production during whole 3-hours period for approximately 100% in standard cultures. Same increase of the glucose production we found in cultured isolated from rats on high saturated fat diet, but without glucagon treatment. However, in cultures isolated from rats on high fat diet glucagon increase of the glucose production was much greater, after first hour 50%, after second hour 75% and after third hour even 100% greater than in standard controls. This progressive increase of the glucose production after glucagon treatment is very important finding which could lead to early hypeglycaemia and insulin

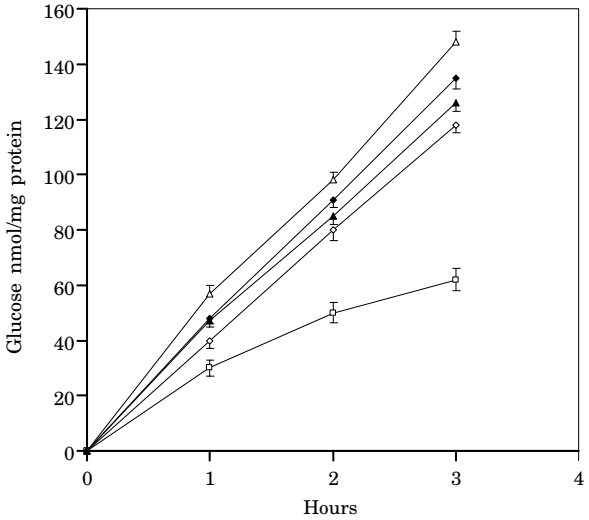

Fig. 1. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium in presence 10 mmol/l pyruvate, isolated from rats on saturated fat diet without (–△–), or treated with insulin (80 nmol/l) (–▲–), or vanadate (1 mmol/l) (–◇–), or insulin and vanadate (–♦–) and standard control (–□–). Each point is the mean ± SEM four five plates in two separates experiments.

resistance (Figure 2). So it is very important to find out how to decrease or prevent further increase of the glucose production from liver. We treated cultures with vanadate using concentration which effectively act in vivo as well as in vitro, but unfortunately without significant effect. Even more, presence of insulin did not influence either glucagon or vanadate effect (Figure 3).

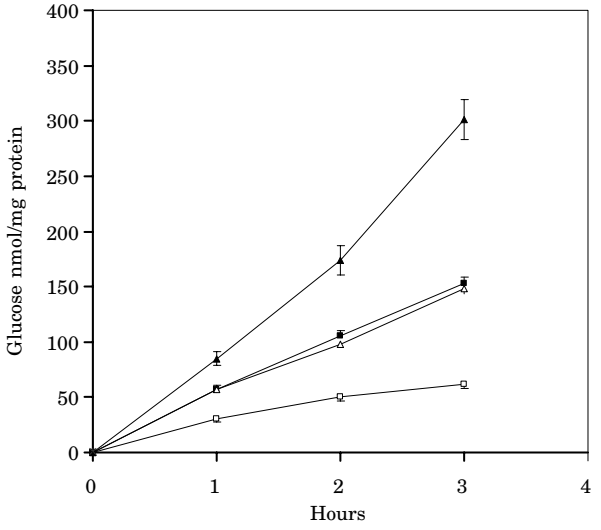

Fig. 2. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium in presence 10 mmol/l pyruvate, isolated from rats on saturated fat diet without (–△–), or treated with glucagon (0,2 μmol/l) (–▲–), comparable with production from rats on standard diet without (–□–), or treated with glucagon (0,2 μmol/l)(–■–). Each point is the mean ± SEM four five plates in two separates experiments.

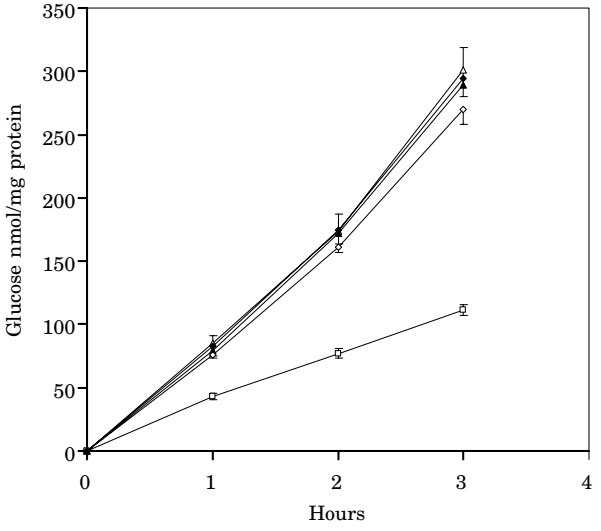

Figure 3. Glucagon stimulated glucose production (nmol/mg prot) isolated from rats on saturated fat diet without (–△–), or treated with insulin (80 nmol/l) (–▲–), or vanadate (1 mmol/l) (–◇–), or insulin and vanadate (–♦–) and standard control (–□–). Each point is the mean ± SEM four five plates in two separates experiments.

Discussion

The present study demonstrated that basic glucose production in hepatocytes isolated from rats on high saturated fat diet was significantly higher comparable with standard control (Figure 1). We already showed similar effect of high unsaturated fat diet on glucose production in cultured hepatocytes[6]. The liver plays a central role in the maintenance of blood glucose homeostasis. This function is achieved by its unique ability to release or remove glucose from the bloodstream depending on the concentration of the hexose and on the hormonal status of the organism. The glucose comes firstly from glycogen. However, the liver glycogen stores are limited and exhausted after few hours of fasting and glucose supply relies then on its synthesis from non-glucidic precursors such as lactate or pyruvate in process of gluconeogenesis. Gluconeogenesis is an energy consuming process. The synthesis of 1 mole of glucose from pyruvate requires 6 moles of ATP[12]. Presumably high hepatic free fatty acid oxidation in hepatocytes isolated in rats on high saturated fat diet promoted gluconeogenesis via production of ATP, NADH, and acetyl-CoA. Fatty acid oxidation played a permissive role on gluconeogenesis and provided the liver with the co-factors necessary for an efficient gluconeogenesis, which could lead to fasting hyperglycemia in vivo. It has becomes conventional wisdom that fasting hyperglycemia is directly related to an increase in hepatic glucose production, but this statement must not to be always correct. Some studies showed that in absolute term hepatic glucose production in patients with diabetes type 2, beside hyperglycemia, are comparable to values in normal individuals[13,14]. This observation does not mean that liver is acting normally responding appropriately on various substrate or hormonal signals. So we tried to find out what is response of cultured hepatocytes in our experimental conditions on glucagon stimulation. We found that this response was inappropriately increased in hepatocytes isolated from rats on high saturated fat diet (Figure 2). Interestingly in subjects with type 2 diabetes was found similar glucagon response on the late stimulation of hepatic glucose output which primarily reflects gluconeogenesis[15].

Second very important finding is very small inhibitory effect of insulin on basic glucose production (Figure 1) and complete lack of effect on glucagon stimulated production (Figure 3). This could be result of insulin resistance, developing after three weeks high fat feeding. The initial stages of type 2 diabetes are characterized by insulin resistance. This leads to the inability of insulin to control the activity of glucogenic enzymes, thereby contributing to an increased hepatic glucose output and elevated blood glucose levels[16]. Also, mice with an organ-specific insulin receptor knockout in the liver show, in addition to a severely impaired glucose tolerance, an increase hepatic glucose production[17]. Therefore, the signaling mechanisms that mediate the regulation of the glucogenic enzymes are of particular interest, because they are potential targets for pharmacological in-

terventions to restore insulin sensitivity to normalize hepatic glucose production. In this regard we used vanadate which has insulin-like and non-insulin-like action[18]. Vanadate alone, as well as in presence of insulin completely normalized basic glucose production and partially prevent glucagon stimulated increase of glucose production in cultured hepatocytes isolated from rats held on high unsaturated fed diet[10]. Unfortunately, in cultures hepatocytes isolated from rats on high saturated fat diet, vanadate significantly decreased glucose production but could not totally normalized basic glucose production and did not influence glucagon stimulated glucose production (Figure 1 and 3). Presently it is not known mechanism of insulin resistance as well as mechanism of vanadate action. Vanadate could act enhancing tyrosine phosphorylation of the insulin receptors or inhibiting of phosphatase[19,20]. There is also another possibility that vanadate act by some other cytosolic or membranous nonreceptor protein tyrosine kinase, as demonstrated in adipocytes[21]. Further studies are required to elucidate the mechanism of vanadate action and insulin resistance in hepatocytes.

Our results clearly demonstrated that possible beneficial effects of vanadate in prevention as well as in therapy of diabetes is dependent on the metabolic status of animal and diet. High saturated fat diet not only increases insulin resistance but also decreases chances of successful therapy.

Acknowledgements

We thank Mr. Boris Tomašević for his expert technical assistance. This study was supported by the grant No. 0065002 from the Croatian Ministry of Science and Technology and by financial support by Zagreb Institute of Public Health.

REFERENCES

1. PEDERSON, E., Am. J. Clin. Nutr., 41 (1985) 1138. — 2. PI-SUNYER, F. X., Ann. Intern. Med., 119 (1993) 655. — 3. O'DOHERTY, R., D. STEIN, J. FOLEY, Diabetologia, 40 (1997) B10. — 4. STORLIEN, L. H., J. A. HIGGINS, T. C. THOMAS, M. A. BROWN, H. Q. WANG, X. F. HUANG, P. L. ELSE, Br. J. Nutr., 83 (2000) S85. — 5. HOWARD, B. V., Ann. N.Y. Acad. Sci., 967 (2002) 324. — 6. ROŠA, J., H. PANCIROV, H. SKALA, A. GADŽIĆ, J. ROŠA, Coll. Antropol., 28 (2004) 631. — 7. STORLIEN, L. H., L. A. BAUR, A. D. KRIKETOS, D. A. PAN, G. J. COONEY, A. B. JENKIS, G. D. CALVERT, L. V. CAMPBELL, Diabetologia, 39 (1996) 621. — 8. HALBERSTAM, M., N. COHEN, H. SHLIMOVICH, L. ROSSETTI, H. SHAMOON, Diabetes, 45 (1996) 659. — 9. ROŠA, J., J. ROŠA, Diabetologia Croatica, 33 (2004) 41. — 10. ROŠA, J., H. PANCIROV, H. SKALA, J. ROŠA, Diabetologia Croatica, 33 (2004) 85. — 11. ROŠA, J., E. RUBIN, Lab. Invest., 43 (1980) 366. — 12. BODEN, G., Life Sciences, 72 (2003) 977. — 13. JENG, C-Y., WH-H. SHEU, MM-T. FULI, Y-DI. CHEN, G. M. REAVEN, Diabetes, 43 (1994) 1440. — 14. HOTHER-NELSEN, O., H. BECK-NIELSEN, Acta Endocrinologica, 124 (1991) 637. — 15. MATSUDA, M., R. A. DEFRONZO, R. GLASS, A. CONSOLO, M. GIORDANO, P. BRESSLER, S. DELPRATO, Metabolism, 51 (2002) 1111. — 16. SALTIER, A. R., Cell, 104 (2001) 517. — 17. MICHAEL, M. D., R. N. KULKARNI, C. POSTIC, S. F. PREVIS, G. I. SHULMAN, M. A. MAGNUSON, C. R. KAHN, Mol. Cell., 6 (2000) 87. — 18. ROŠA, J., J. ROŠA, Coll. Antropol., 28 (2004) 309. — 19. NECHAY, B. R., L. B. NANNINGA, P. S. E. NECHAY, Federation Proc., 45 (1986) 123. — 20. TAMURA, S., T. A. BROWN, J. H. WHIPPLE, J. Biol. Chem., 259 (1984) 6650. — 21. ELBERG, G., Z. HE, J. LI, N. SEKAR, Y. SHECHTER, Diabetes, 46 (1997) 1684.

J. Roša

Department of Physiology, School of Dental Medicine, University of Zagreb, PO Box 978, Šalata 3, Zagreb 10000, Croatia, e-mail: jrosa@mef.hr

UČINCI VANADATA NA STVARANJE GLUKOZE U KULTIVIRANIM HEPATOCITIMA IZOLIRANIM U ŠTAKORA NA DIJETI BOGATOJ ZASIĆENIM MASTIMA

SAŽETAK

Inzulinska rezistencija je zajednički fenomen koji se pojavljuje u pretilosti i dijabetesu tipa 2. Zajednički faktor koji je važan za razvoj dijabetesa i inzulinske rezistencije je uzimanje zasićenih masti. Vanadate poboljšava glukoznu homeostazu in vivo. Svrha ovog rada je otkriti promjene jetrenog stvaranja i otpuštanja glukoze u ovisnosti o dijeti bogatoj zasićenim mastima i moguće direktno djelovanje vanadate u kultiviranim hepatocitima. Hepatociti su izolirani pomoću kolagenaze i kultivirani 24 sata u M 199 mediju bez dodatka seruma. Stvaranje glukoze u hepatocitima izoliranim iz štakora na visoko masnoj dijeti bogatoj zasićenim masnoćama bilo je 139% veća u usporedbi s standardnom kontrolom. Glukagon 100% povećava proizvodnju glukoze u hepatocitima dobivenim iz štakora na standardnoj dijeti, a 200% u štakora na dijeti sa zasićenim masnoćama. Vanadate je signifikantno smanjio bazičnu proizvodnju glukoze, a nije utjecao na glukagonom stimuliranu produkciju glukoze. Prisutnost inzulina nije utjecala na glukagonski kao ni na vanadate efekt. Dijeta bogata zasićenim masnoćama ne povećava samo inzulinsku rezistenciju nego također smanjuje izglede za uspješno liječenje dijabetesa.