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Effects of Troglitazone and Insulin on Glucose Production in Cultured Hepatocytes Isolated from Rats on High Fat Diet

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A B S T R A C T

Increased dietary fat intake in general, and saturated fat specifically, will lead to the impairment of insulin action. The aim of this study was to find out the changes in hepatic glucose output in dependence of fat diet and a possible direct action of insulin and trogitazone in 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 fat diet (unsaturated fat) was 79% higher compared to control and even 139% higher than in rats on high-fat diet (saturated fat). Troglitazone significantly decreased the glucose production in hepatocytes obtained from rats on unsaturated fat diet. The troglitazone in presence of insulin totally normalized glucose production but also only in hepatocytes obtained from rats on unsaturated-fat diet. The troglitazone showed an insulinomimetic as well as insulin-sensitizing effect but only in rats on unsaturated-fat diet.

Key words: *insulin, insulin resistance, glucose production, gluconeogenesis, troglitazone*

Introduction

Insulin resistance is a common phenomenon in obesity and Type 2 diabetes[1]. A high level of dietary fat intake is believed to be a major factor in the development of obesity and insulin resistance[2]. The pathophysiology of Type 2 diabetes involves characteristic defects in three main organ systems that conspire together to produce abnormal glucose metabolism. Metabolic defects in the liver, the peripheral target

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tissues and the pancreatic β-cells all contribute to the syndrome. The enhancement of insulin action might be an effective pharmacological approach to diabetes Type 2. Troglitazone appears to enhance the insulin action without directly stimulating insulin secretion. This drug may reduce plasma glucose by facilitating glycolysis and also by inhibiting gluconeogenesis in the liver and this is involved in the enhancement of insulin action both in vivo as well as in vitro[3–5].

Fat feeding induces insulin resistance firstly in liver followed by a widespread impairment of insulin action in white and brown adipose tissue and skeletal muscle with early metabolic changes favoring an oversupply of energy substrate to skeletal muscle[6]. The type of dietary fat is thought to be important in this regard. Increased dietary fat intake in general, and saturated fat specifically, will lead to increased adiposity. This increased adiposity, particularly central adiposity, will lead to the impairment of insulin action. However, increased fat intake also appears to lead to insulin resistance independent of adiposity. Again saturated fat, in particular, would appear to be a major culprit. Increased hepatic glucose output correlates well with fasting glucose levels and is the main cause of fasting hyperglycemia in Type 2 diabetes[2]. We tried to find out if the increase of hepatic glucose output depends on the percentage of saturated and unsaturated fat consumed during the early experimental period before the development of manifest diabetes symptoms. We measured the rate of glucose production in hepatocytes isolated from rats fed with saturated fat of mainly animal origin and unsaturated fat from sunflower oil and we found striking differences in the rate of glucose production as well as in the insulin and trogitazone action.

Materials and Methods

In all experiments, male adult Wistar rats, each weighting 187–240 g, were used. Rats were housed individually in wire cages in a temperature-controlled room (21 ± 1 °C), on 12h light-dark cycle, with free access to food and water for three weeks. In terms of energy, the standard food contained 57% carbohydrates, 32% protein and 11% fat. The high-fat diet was prepared by mixing 30% of sunflower oil or 30% of animal fat with standard food. In both cases the high-fat diet contained 30% carbohydrates, 16% protein and 54% fat (Table 1).

The principles of animal care (NIH publication No. 85–23, revised 1985) were applied.

The hepatocytes were isolated by a modified collagenase-perfusion technique[7]. 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 a measure of viability. After washing twice with the same collagenase-free medium, the cells were suspended in a final concentration of one million

TABLE 1
COMPOSITION OF DIETS

	Standard food	Sunflower oil 30%	Animal fat 30%
Carbohydrates(%of energy)	57	30	30
Protein (% of energy)	32	16	16
Fat (% of energy)	11	54	54

cells per ml M199 serum-free medium. Three ml of cell suspension were placed in 60-mm Petri dishes previously coated with collagen. The 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 by a fresh medium 4 hours later to remove the unattached cells while the hepatocytes were incubated for the next 24 hours in the M199 serum-free medium.

After having been immersed for 24h in the culture, the medium was removed and the cells were incubated in glucose-free Hanks-Hepes medium, containing 10 mmol/l pyruvate, without hormones (control), either with insulin (80 nmol/l) or with troglitazone (2 mg/l). The glucose production was measured by incubating the cultures in glucose-free Hanks-Hepes medium with the addition of 10 mmol/l pyruvate. The glucose was determined enzymatically by means of glucose oxidase. The incubation medium was removed and the hepatocytes were washed three times with cold saline and immediately frozen in liquid nitrogen. The cells were digested in 0.2 N NaOH and an aliquot was taken for the determination of glycogen and protein. The amount of protein was determined by the Lowry et al. method[8].

Albumin bovine, glutamine, HEPES, M199 medium, Swim's S-77 medium, insulin, were obtained from Sigma; collagenase CLS II (131U/ mg) was purchased from Worthington; collagen R was purchased from Serva; troglitazone (Rezulin) was purchased from Parke-Davis.

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 $NaHCO_3$.

The data are expressed as means ± SEM. The statistical significance was evaluated by Student's t-test. $P<0.01$ was considered statistically significant.

Results

The dietary intake expressed in terms as gram per day was significantly higher in rats on standard food compared with animals on high-fat diet, mostly unsaturated (sunflower oil) as well as in animal on rendered fat diet (Table 2). There was no significant difference in energy intake between the three groups over a 21-day period. The final body weight was a little, but not significantly higher in rats on high-fat diet.

TABLE 2
METABOLIC CHARACTERISTIC OF RATS IN EACH GROUP

Characteristic	Standard food	Unsaturated fat 30%	Saturated fat 30%
No. of animals	3	3	3
Diet duration (d)	21	21	21
Final body weight (g)	297 + 18*	305 + 24**	309 + 22**
Food intake (g/d)	23,1 + 2*	18,4 + 1,1***	18,7 + 1,6***
Energy intake (KJ/rat.day)	364 + 31*	430 + 26**	437 + 37**

* Mean + SEM
** Difference from values in group 1, N.S.
*** Difference from values in group 1, $p < 0,01$

The highest rate of glucose production in control cultures occurred in the first hour, whereas a considerably lower rate was found during the second hour and it completely stopped in the third hour (Figure 1). Since these cells were incubated in glucose-free Hanks-Hepes medium without pyruvate, the glucose production in control cultures was delivered by quick activation of glycogenolysis. The highest rate of glycogenolysis was in the first hour, whereas a considerably lower rate was found during the second hour and it completely stopped in the third hour. In cultured hepatocytes isolated from the rats on standard diet, the rate of glucose production was significantly higher in the presence of 10 mmol/l pyruvate, compared with the control group. This difference was the highest in the third hour (140%) as a result of a strong activation of gluconeogenesis (Figure 1). The addition of pyruvate very strongly (427%) increased the glucose production in hepatocytes isolated from rats on high-fat (sunflower oil) diet. There was an almost linear increase in glucose production throughout the 3-hour period. The increase in glucose production was even higher (569%) in cultures isolated from rats on high animal-fat diet (Figure 1).

The insulin alone, in a concentration of 80 nmol/l, did not change the glucose production in hepatocytes isolated from rats on high-fat (sunflower oil) diet. The glucose production remained 79% higher compared with standard cultures (Figure 2). The addition of troglitazone (2 mg/l) significantly decreased the glucose production, but it was still 48% higher than

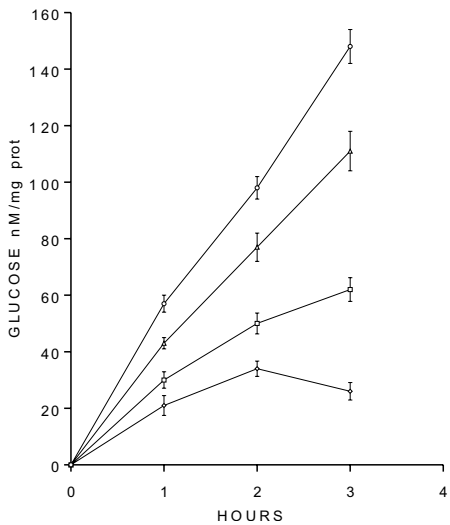

Fig. 1. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium without (control) (-◊-) or in the presence of 10 mmol/l pyruvate, isolated from rats standard (-□-), sunflower oil (-△-) and animal-fat diet (-○-). Each point is the mean ± SEM for nine plates in three separates experiments.

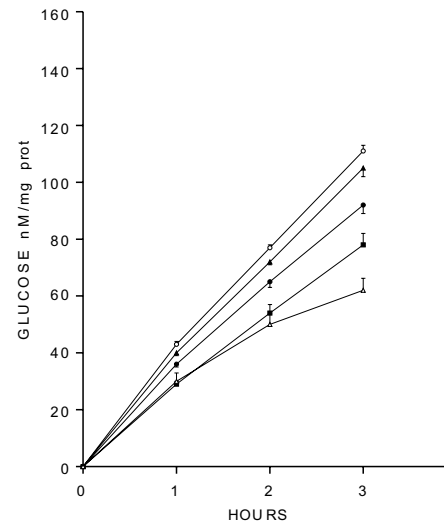

Fig. 2. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium in presence 10 mmol/l pyruvate, isolated from rats on sunflower oil diet without (-○-), or treated with insulin (80 nmol/l) (-▲-), or troglitazone (2 mg/l) (-●-), or insulin and troglitazone (-■-) and standard control (-△-). Each point is the mean ± SEM for nine plates in three separate experiments.

in standard cultures. However, troglitazone in cultures treated with insulin completely normalized the glucose production in the first and second hour of incubation. Later, during the third hour, the glucose production was minimally (25%) but significantly higher than in standard cultures (Figure 2).

On the other hand, in hepatocytes isolated from rats on high animal fat diet, the basic glucose production was 139% higher than in standard cultures. The insulin only minimally but significantly decreased this production, but it still remained 103% higher than in standard cultures. However, troglitazone alone or in combination with insulin could not normalize the glucose production and it remained 114% and 129% higher than in standard cultures (Figure 3).

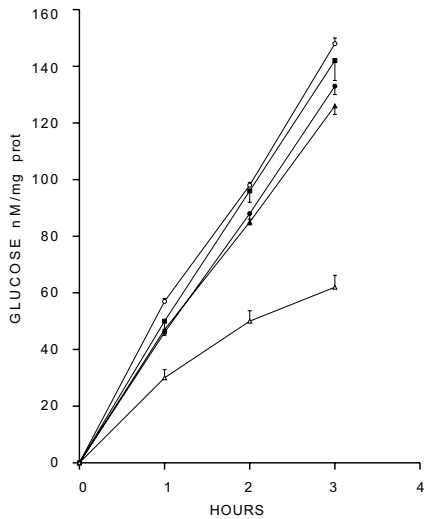

Fig. 3. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium in the presence of 10 mmol/l pyruvate, isolated from rats on animal fat diet without (-○-), or treated with insulin (80 nmol/l) (-▲-), or troglitazone (2 mg/l) (-●-), or insulin and troglitazone (-■-) and standard control (-△-). Each point is the mean ± SEM for nine plates in three separates experiments.

Discussion

The present study demonstrates that the basic glucose production in hepatocytes cultured in vitro is highly dependent on the percentage of fat consumed during the three-week period in rats. It is also dependent on the percentage of saturated fat in diet, independently of adiposity since final body weight was not significantly different at the end of the experiment. A good deal of evidence has now accumulated to show that the fatty acid profile of the diet is important. The evidence has ranged from early in vitro evidence of differences in insulin binding and glucose transport in cells incubated with different types of fat or in vivo in animals on different fat diets[9–12]. We showed that the glucose production in hepatocytes isolated from rats on high-fat (sunflower oil) diet was 79% higher compared with standard control and even 139% higher in rats on high-saturated-fat diet. Probably elevated free fatty acids contribute to the increased rates of gluconeogenesis. This is an important finding because increased hepatic glucose output correlates well with fasting glucose levels and is the main cause of fasting hyperglycemia in Type 2 diabetes[2]. Hyperglycemia in the early period along with elevated free fatty acid levels also contributes to the development of insulin resistance[1,13]. Hepatic free fatty acid oxidation promotes gluconeogenesis through the production of ATP, NADH, and acetyl-CoA (to activate pyruvate carboxylase) [14].

The failure of insulin to inhibit hepatic glucose production is a cardinal feature of insulin resistance in the liver and a major factor contributing to hyperglycemia in diabetic states. Developing of insulin resistance could provide a simple explanation to our results since insulin did not decrease the glucose production in hepatocytes in either experimental group. Some in vivo studies suggested that much

of insulin action on the liver may be indirect and related to systemic rather than hepatic insulin effects[15,16]. We already showed that insulin does not decrease the glucose production in hepatocytes isolated from rats on a standard diet and cultured in vitro in a glucose-free medium[17]. This result is not so unexpected since, when insulin is injected into an animal in vivo, it causes a deep hypoglycemia, which in turn initiates an intense glycogenolysis in the liver and increases the glucose production[18]. When blood glucose concentration is low in vivo or in glucose-free medium in vitro, the hepatocytes produce glucose mainly from glycogen, which was also demonstrated. The glycogen stores are limited and get exhausted after one hour in cultured hepatocytes. The glucose supply relies then on its synthesis from non-glucidic precursors such as lactate or pyruvate. The process of gluconeogenesis was activated during the first hour of incubation in our experiments, but it became the main process for glucose production during the second and the third hour and again the insulin could not decrease the glucose production in a glucose-free medium. In elegant experiments performed on LIRKO mice Fisher & Kahn showed that an intact insulin-signaling pathway in the liver is required for insulin direct and indirect action on hepatic glucose production[19]. So with all these facts in mind we concluded that insulin resistance was probably developed during the three-week period of high-fat feeding.

Thiazolidinediones appear to enhance the insulin action reducing the insulin resistance. Troglitazone is one of these insulin-sensitizing agents[3]. Primary tissue target for troglitazone action is peroxisome proliferator activated receptor-γ (PPAR-γ) in fat. Alternatively it may have direct actions on PPAR-γ in other tissues like muscle and liver[20]. This is in agreement with our finding that troglitazone decreased the glucose production in hepatocytes acting directly in culture but only in rats on sunflower-oil diet. It is important to say that this effect of troglitazone was observed in the total absence of insulin. It was already shown that troglitazone reduced gluconeogenesis in the hepatocytes obtained from fasted rats and not in those from fed animals[21]. These results cannot explain the differences in troglitazone action in our two experimental groups. Anyhow, the troglitazone in the presence of insulin totally normalized the glucose production but again only in hepatocytes obtained from rats on sunflower-oil diet. Presumably insulin resistance in our two experimental groups is not the same. Etiological factors are different (saturated and unsaturated fat). The mechanism of insulin resistance could also be different, as well as sensitivity to the therapeutic action of troglitazone. Our results clearly demonstrate that the result and success of therapy of diabetes Type 2 is dependent on the metabolic status of animals and on diet.

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REFERENCES

1. O'DOHERTY, R., D. STEIN, J. FOLEY, Diabetologia, 40 (1997) B10. — 2. 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. — 3. SALTIEL, A. R., J. M. OLEFSKI, Diabetes, 45 (1996) 1661. — 4. HORIKOSHI, H., T. FUJIWARA, M. SHIMADA, S. YOSHIOKA, T. TOKUI, T. YOSHIOKA, Diabetes, 39 (1990)

111A. — 5. RAMAN, P., S. E. FOSTER, M. C. STOKES, J. K. STRENGE, R. L. JUDD, Life Sciences, 62(1998) PL89. — 6. KRAEGEN, E. W., P. W. CLARK, A. B. JENKIS, E. A. DALEY, D. J. CHISHOLM, L. H. STORLIEN, Diabetes, 40 (1991) 1397. — 7. ROŠA, J., J. ROSA, Coll. Antropol., 28 (2004) 309. — 8. LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, R. J. RANDEL, J. Biol. Chem., 193 (1951) 265. — 9. GRUNFELD, C., K. BAIRD, C. R. KAHN, Biochem. Biophys. Res. Commun., 103 (1981) 219. — 10. YOREK, M., E. LEENEY, J. DUNLAP, B. GINSBERG, Inv. Ophth. Vis. Sci., 30 (1989) 2087. — 11. STORLIEN, L. H., E. W. KRAEGEN, D. J. CHISHOLM, G. L. FORD, D. G. BRUCE, W. S. PASCOE, Science, 237 (1987) 885. — 12. STORLIEN, L. H., A. B. JENKIS, D. J. CHISHOLM, W. S. PASCOE, S. KHOURI, E. W. KRAEGEN, Diabetes, 40 (1991) 280. — 13. BODEN, G., Diabetes, 46 (1997) 3. — 14. WILLIAMSON, J. R., R. A. KRESBERG, P. W. FELTS, Proc. Natl. Acad. Sci. USA, 56(1966) 247. — 15. ADER, M., R. N. BERGMAN, Am. J. Physiol., 245 (1990) E1020. — 16. MITTELMAN, S. D., Y. Y. FU, K. REBRIN, G. M. STEIL, R. N. BERGMAN, J. Clin. Invest., 100 (1997) 3121. — 17. ROŠA, J., J. ROSA, Diabetologia Croat., 33 (2004) (in press). — 18. HERS, H. G., Ann. Rev. Biochem., 45 (1976) 167. — 19. FISHER, S. J., C. R. KAHN, J. Clin. Invest., 111 (2003) 463. — 20. SPIEGELMAN, B. M., Diabetes, 47 (1998) 507. — 21. RAMAN, P., S. E. FOSTER, M. C. STOKES, J. K. STRENGE, R. L. JUDD, Life Sci., 62 (1997) 89.

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UČINCI TROGLITAZONA I INZULINA NA PROIZVODNJU GLUKOZE U KULTIVIRANIM HEPATOCITIMA IZOLIRANIM IZ ŠTAKORA NA DIJETI BOGATOJ MASTIMA

S A Ž E T A K

Povećani unos masnoća, a posebno zasićenih masti, dovodi do smanjenja inzulinskog učinka. Cilj istraživanja je otkriti promjene u proizvodnji glukoze iz jetre u ovisnosti o masnoj dijeti i moguće direktne učinke inzulina i troglitazona u hepatocitima. Hepatociti su izolirani pomoću kolagenaze i kultivirani 24 sata u M 199 mediju bez dodatka seruma. U hepatocitima izoliranim iz štakora na visoko masnoj dijeti (nezasićene masnoće) proizvodnja glukoze bila je 79% veća u usporedbi sa kontrolom i 139% veća ako su bili na visoko masnoj dijeti (zasićene masnoće). Troglitazon je signifikantno smanjio proizvodnju glukoze u hepatocitima dobivenim iz štakora na masnoj dijeti sa nezasićenim masnoćama. Troglitazon je u prisutnosti inzulina u potpunosti normalizirao proizvodnju glukoze ali opet samo u štakora koji su hranjeni na masnoj dijeti sa nezasićenim masnoćama. Troglitazon je sam djelovao slično inzulinu, a osim toga povećao je osjetljivost stanica na inzulin, ali samo ako su štakori bili na visoko masnoj dijeti sa nezasićenim masnoćama.