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EFFECTS OF CHRONIC BENFLUOREX TREATMENT ON THE ACTIVITIES OF KEY ENZYMES OF HEPATIC CARBOHYDRATE METABOLISM IN OLD SPRAGUE–DAWLEY RATS

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Abstract—Chronic effects of benfluorex on some parameters of carbohydrate metabolism have been studied in 24-month-old Sprague–Dawley rats. Treatment once a day for 14 days with 25 mg benfluorex per kg body weight lowered body weight, decreased circulating insulin and resulted in an increase in hepatic glycogen. Measurement of the activities of several important regulatory enzymes of hepatic carbohydrate metabolism showed a significant decrease in the activities of phosphoenolpyruvate carboxykinase and glycogen phosphorylase. The activity of glucose-6-phosphatase, on the other hand, was slightly increased. Taken collectively, our data offer an explanation for the observed inhibition of hepatic glucose production by chronic benfluorex treatment in cases of hyperinsulinemia.

Benfluorex (1-(3-trifluoromethylphenyl)-2-[*N*-(2-benzyloxy-ethyl)amino]propane) is currently used as a hypolipidemic agent and is known to lower both plasma triacylglycerol and cholesterol levels [1]. In addition, the drug has been shown to affect carbohydrate metabolism. The mechanism of action of this compound is far from understood. In recent animal studies [1, 2], chronic administration of benfluorex was found to decrease hyperinsulinaemia and insulin resistance and to lower an existing hyperglycemia. In isolated hepatocytes gluconeogenesis was inhibited following treatment of the cells with benfluorex [3, 4]. The biochemical basis for this effect on carbohydrate metabolism is not yet clear.

The present experiments were mainly designed to study the effects of benfluorex on several key enzymes of hepatic carbohydrate metabolism. Attention has been focused on the chronic effects of the drug, because, these are of most interest from a therapeutic point of view. Old Sprague–Dawley rats are hyperinsulinemic and show a decreased peripheral sensitivity to insulin and are, therefore, often used as a model for studies on insulin resistance. In this model, benfluorex was shown to diminish the elevated level of insulin [5]. Furthermore, the drug appears to improve the peripheral insulin sensitivity [6].

Here we present evidence that long-term treatment with benfluorex lowers the activity of phosphoenolpyruvate carboxykinase (PEPCK†), a major regulatory enzyme of gluconeogenesis. In addition, it was found that in liver the activity of glycogen phosphorylase is decreased and that the level of glycogen is increased. Collectively, our observations provide an explanation for the observed decrease in hepatic glucose production by chronic benfluorex treatment in cases of hyperinsulinemia.

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† Abbreviations: PEPCK, phosphoenolpyruvate carboxykinase; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

MATERIALS AND METHODS

Animal model and treatment. Male Sprague–Dawley rats (530–740 g, 24 months-old) were purchased from Iffa Credo (Brussels, Belgium). After arrival they were allowed 2 weeks to become accustomed to their new environment, individual housing and an inverted light/dark rhythm. In this period, their body weight did not change. Under anaesthesia the animals were then equipped with a permanent stomach cannula. This silicon cannula was channelled subcutaneously to a head socket. After 1 week recovery, this head socket was connected to the outside of the cage via a swivel, which allowed the animals to move freely and permitted injections into the stomach without further handling of the animals. In this way administration of benfluorex or carrier caused no stress, and even went unnoticed by the rat. Pilot studies had shown that oral forced feeding was impossible with these very old Sprague–Dawley rats without causing considerable stress.

Water and food (normal lab chow) were available *ad libitum* to the rats throughout the experiment. In the week of recovery after surgery, drinking water consisted of 2.5% (w/v) glucose in saline (0.9%, w/v). Subsequently, this was replaced by normal water and 6 days later drug administration started. One group of seven rats received benfluorex (12.5 mg/mL in 1% methylcellulose; 25 mg benfluorex per kg body weight) and the other group of seven rats received an equivalent amount of methylcellulose only (control group). This administration occurred daily at 9.00 a.m., just before the dark period started, and hence, just prior to the normal eating

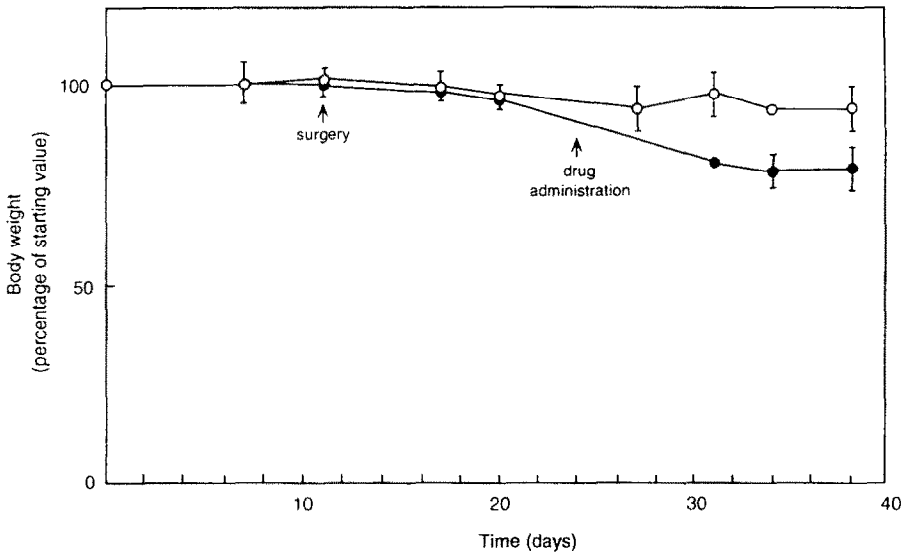

Fig. 1. Profile of body weight of control (○) and benfluorex-treated (●) old Sprague–Dawley rats. Day 0: arrival of the animals in the laboratory; average starting weight 686 ± 54 g for the benfluorex-treated group and 643 ± 23 g for the control group. Arrows indicate: time of surgery for installing permanent stomach cannula and starting time of administering 1% methylcellulose alone or benfluorex in 1% methylcellulose. From day 18 on, the supporting glucose was omitted from the drinking water. Results are expressed as the means ± SD of the percentage of the starting value of each animal in the two groups (each group consisted of seven rats).

time of the animals. This regime was continued for 14 days. Twenty-four hours after the final administration of the drug, the animals were weighed and blood was taken via an orbital puncture under light ether anaesthesia. The rats were then immediately decapitated and their livers were quickly isolated.

Homogenization. Homogenates (20%, w/v) of the excised livers were prepared immediately by mincing at 0° with an Ultra Turrax (3 pulses of 15 sec). For analyses of liver glycogen levels, glycogen phosphorylase and glycogen synthase activities, part of the liver was homogenized in buffer (pH 7.6, 0°) containing: 50 mM β-glycerophosphate, 10 mM EDTA, 1.4 mM EGTA, 50 mM NaF, 10 mM β-mercaptoethanol, 100 mM NaCl and 0.25% (w/v) oyster glycogen. The homogenate was centrifuged at 4° for 10 min at 500 *g*. Homogenates for all other determinations of enzyme activities and protein contents were prepared in buffer (pH 8.0, 0°) containing: 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 5 mM β-mercaptoethanol. This homogenate was centrifuged at 4° for 30 min at 48,000 *g*. The supernatants were filtered through gauze and used immediately (glucose-6-phosphatase and pyruvate kinase assays) or stored in aliquots at −70° until use.

Assays. Glycogen synthase (EC 2.4.1.11) activity was determined by the incorporation of [^{14}C]UDP-glucose into glycogen in the presence of 10 mM glucose-6-phosphate via a combination of the methods of Thomas *et al.* [7] and Villa-Moruzzi *et al.* [8]. The addition of glucose-6-phosphate results in the measurement of total activity, not only the active, dephosphorylated form. Glycogen phosphorylase (EC 2.4.1.1) activity was measured by the incorporation of [U-^{14}C]glucose-1-phosphate into glycogen [9]. In the assay for glycogen phosphorylase perforce only the active, phosphorylated form is measured; the presence of fluoride and EDTA in the homogenization buffer preserved the phosphorylation state of the enzyme.

The activities of fructose-1,6-bisphosphatase (EC 3.1.3.11), pyruvate kinase (EC 2.7.1.40), glucokinase (EC 2.7.1.2) and lactate dehydrogenase (EC 1.1.1.27) were determined spectrophotometrically as described in Refs. 10, 11, 12 and 13, respectively.

Glucose-6-phosphatase (EC 3.1.3.9) was determined by monitoring the production of labelled glucose from [U-^{14}C]glucose-6-phosphate as described [14, 15]. PEPCK, (EC 4.1.1.32) was assayed by the incorporation of [^{14}C]$NaHCO_3$ as described [16].

Phosphofructokinase (EC 2.7.1.11) was assayed spectrophotometrically as described previously [17]. The activity was determined in the presence of 1 μM fructose-2,6-bisphosphate to ensure maximal activity.

All enzyme assays were performed at 25°, in duplicate. Statistical analysis was performed via Student's *t*-test.

Protein was determined using the Lowry method with bovine serum albumin as standard [18]. Glycogen content of the livers was assayed by precipitation of the glycogen from the homogenate on filter paper [19]. After washing, the glycogen was hydrolysed enzymatically, glucose was determined and corrections were made for glycogen present in

Table 1. Effects of benfluorex treatment on body weight, blood parameters and liver glycogen

Parameters after 2 weeks of treatment	Control	Benfluorex
Glucose (mM)	8.3 ± 1.1	8.5 ± 0.6
Insulin (μU/mL)	18.4 ± 6.2	11.5 ± 3.6*
Liver protein (mg/g wet wt)	160 ± 15	130 ± 12†
Glycogen (μg/mg protein)	202 ± 57	385 ± 148†
Body weight (g)	610 ± 22	521 ± 46‡

The results are expressed as means ± SD for the seven rats in each group. Significantly different from control, treated with methylcellulose carrier only: *$P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

the homogenization buffer. Glucose was assayed using hexokinase and glucose-6-phosphate dehydrogenase. Plasma glucose was determined by the GOD-Perid method. Insulin levels were measured with a radioimmunoassay kit from Pharmacia (Uppsala, Sweden).

Sources of materials. [U-^{14}C]glucose, [^{14}C]-$NaHCO_3$, [^{14}C]UDP-glucose and [U^{14}C]glucose-1-phosphate were from New England Nuclear (Boston, MA, U.S.A.). All enzymes and fine chemicals were from Boehringer (Mannheim, Germany) or the Sigma Chemical Co. (St Louis, MO, U.S.A.). Benfluorex was supplied by Servier (Paris, France).

RESULTS

Effects of benfluorex treatment on body weight

After surgery for the placement of a permanent stomach cannula we observed in all individual animals a slight loss of body weight. When glucose, which was administered to assist in recovery from surgery, was omitted from the drinking water a small further drop in body weight was noticed.

Benfluorex treatment caused a significant loss in body weight compared to the controls (Fig. 1). This loss in body weight occurred in the first week of drug treatment. No further decrease was observed from day 7 onward.

Effects of benfluorex treatment on the levels of liver glycogen, blood glucose and insulin

Administration of benfluorex for 14 days significantly decreased the concentration of insulin in the circulation. The level of blood glucose, however, was unaltered by this treatment (Table 1). In animals presented with benfluorex the amount of glycogen in liver was almost doubled as compared to the control animals (Table 1).

Effects of benfluorex treatment on hepatic enzymes

The influence of 14 day treatment of animals with benfluorex on the activity in liver of a number of key enzymes of carbohydrate metabolism is presented in Table 2. The specific activity of most enzymes determined in this study was unaffected, but the activities of glycogen phosphorylase and PEPCK, major regulatory enzymes of glycogen metabolism and gluconeogenesis, respectively, were diminished in the drug-treated animals. The specific activity of glucose-6-phosphatase, on the other hand, was slightly increased (Table 2).

The protein content per gram liver of the treated group was 19% less than that of the control group (Table 1). Therefore, the values for the different enzyme activities were not only expressed on the basis of protein content but also in a way independent of that parameter. For that purpose the enzyme activities were also expressed per unit lactate dehydrogenase activity. The reason for doing so is that the latter enzyme is considered to be a constitutive enzyme without changes in expression caused by alterations in metabolic conditions. If the data are expressed in the latter way only PEPCK differs significantly between the two sets of animals (Table 2). The fact that then the activities of glycogen phosphorylase and glucose-6-phosphatase were no longer statistically different from those of the control animals could be the result of the large variation in the specific activities of lactate dehydrogenase (Table 2). The large variation in specific activities of the latter enzyme was caused by large differences in the activities of the various animals in each group (up to 37%) and was not the result of a large variation in the assay of each animal (less than 2%).

A pilot study in our laboratory with one week benfluorex treatment of 3-month-old Sprague–Dawley rats also revealed a decreased activity level of glycogen phosphorylase (not shown). The other hepatic enzymes determined in the present study were, however, unaffected in that study on young Sprague–Dawley rats. This indicates that the decreased glycogen phosphorylase activity after benfluorex treatment is a general phenomenon that does not only occur in the old Sprague–Dawley rats.

DISCUSSION

Benfluorex, mainly used as a hypolipidemic agent, is also known to decrease hyperinsulinaemia and insulin resistance. Insulin resistance and elevated insulin levels are a characteristic finding in both simple obesity and non-insulin dependent diabetes mellitus. Old Sprague–Dawley rats are a suitable animal model of insulin resistance. In the present study treatment of 24-month-old Sprague–Dawley rats with 25 mg benfluorex per kg body weight for 14 days indeed resulted in a decrease in circulating insulin levels.

Table 2. Effects of chronic benfluorex treatment on the activity of some hepatic enzymes

Enzyme	Control (nmol/min/mg protein)	Benfluorex (nmol/min/mg protein)	Control (nmol/min/unit LDH)	Benfluorex (nmol/min/unit LDH)
LDH	4990 ± 1000	5330 ± 700	1	1
Synthase	17.22 ± 1.99	16.34 ± 2.41	3.67 ± 1.35	3.29 ± 0.87
Phosphorylase	391.10 ± 28.90	327.00 ± 64.30*	81.82 ± 21.51	62.64 ± 16.99
Glucokinase	11.93 ± 3.38	13.78 ± 2.34	2.58 ± 1.30	2.64 ± 0.69
PFK	15.25 ± 1.84	16.87 ± 1.08	3.14 ± 0.63	3.23 ± 0.61
PK	77.80 ± 18.40	83.00 ± 18.30	16.50 ± 6.32	16.15 ± 6.11
PEPCK	5.60 ± 1.10	3.29 ± 0.93†	1.13 ± 0.16	0.61 ± 0.16‡
FBPase	77.31 ± 13.15	68.90 ± 8.25	15.82 ± 3.10	13.07 ± 1.77
G6Pase	1.68 ± 0.39	2.23 ± 0.51*	0.34 ± 0.07	0.43 ± 0.13

Abbreviations: LDH, lactate dehydrogenase; synthase, glycogen synthase; phosphorylase, glycogen phosphorylase; PFK, phosphofructokinase; PK, pyruvate kinase; FBPase, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase.

Enzyme activities in columns 1 and 2 represent means ± SD of the seven animals in each group. In columns 3 and 4 the enzyme activities are expressed per unit (μmol/min) LDH.

Significantly different from control: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Benfluorex treatment of old Sprague–Dawley rats also significantly decreased body weight. The effect represented a decrease of about 15%. This reduction in body weight is comparable to the weight loss seen in JRC-LA-corpulent rats treated with the same amount of the drug [2]. In both studies the maximal weight loss stabilized after about 1 week of drug administration. From a therapeutic point of view the drop in body weight may be a bonus of benfluorex treatment.

Animal studies [1, 2] as well as experiments with isolated hepatocytes [3, 4] revealed effects of benfluorex on carbohydrate metabolism. However, there is a major difference between the two types of experiments. In the animal studies, changes due to chronic administration of the drug were studied whereas the work with the isolated cells explored acute effects of the drug. In the present investigation the focus was on chronic effects. To eliminate short-term effects, the animals were killed 24 hr after the last administration of benfluorex. Therefore, the changes observed in the activities of some of the enzymes are of a long-term nature and are probably the result of changes in enzyme level rather than in the activity of pre-existing enzyme molecules. However, immunochemistry using antibodies raised against these enzymes will be required to distinguish between these two possibilities.

The fall in the activity of PEPCK following chronic benfluorex treatment (Table 2) most likely decreases the capacity of the gluconeogenic pathway resulting in less generation of glucose by the liver. This decrease in gluconeogenic capacity is even larger when related to the whole animal, because it is known that the decrease in body weight after benfluorex treatment is accompanied by a decrease in liver weight [20, 21], and also by a decrease in protein content per gram of liver (Table 1). Gluconeogenesis is not only important during starvation or in diabetes mellitus but is also operative in non-diseased and well-fed conditions. In the latter situation the pathway is very active for the synthesis of glucose or glycogen since a major portion of dietary glucose reaches the circulation as lactate [22]. Apparently the observed decrease in gluconeogenic capacity did not result in a decrease in hepatic glycogen levels, as these were almost doubled after treatment. It should be realized, however, that hepatic glycogen levels are determined by a complex set of regulatory mechanisms, resulting in varying rates of both synthesis and degradation.

The decrease in hepatic glycogen-phosphorylase activity following long-term treatment with benfluorex (Table 2) works in the same direction as the fall in activity of PEPCK, i.e. reduction in glucose output by the liver. The effect of benfluorex on the activity of glycogen phosphorylase is further substantiated by the observation that under that condition the hepatic level of glycogen is increased. The decreased glucose-generating capacity of the liver did, however, not result in a noticeable effect on the blood-glucose concentration. The possible relationship between these two observations is still unclear. It should be realised, however, that both long- and short-term effects are involved in the complicated process of glucose homeostasis. The present study corroborates earlier studies in which plasma glucose concentrations were unchanged upon chronic treatment with benfluorex. A decrease in circulating glucose was only observed during a glucose challenge [see Ref. 1]. In the light of the above observations, benfluorex must be considered an antihyperglycemic rather than a hypoglycemic agent.

Our studies demonstrate that the previously observed normalization of hepatic glucose production is accompanied by a long-term decrease in gluconeogenic capacity (PEPCK) and glycogen degradation (glycogen phosphorylase). It is not yet known, however, whether these decreased enzyme levels are, (1) a direct effect of benfluorex on the expression of PEPCK and glycogen phosphorylase, or (2) an indirect effect of benfluorex, acting for instance via an increased insulin sensitivity [23]. In case (1) the reduced expression of PEPCK and glycogen phosphorylase would lead to a diminished

glucose output by the liver. This will result in an improved insulin sensitivity [Blondel *et al.* cited in Ref. 1]. In this way the observed antihyperglycemic effect of benfluorex could be the result of its action on the expression of PEPCK. It is, however, also possible that the observed effect of benfluorex on hepatic glucose production is a secondary one (2), whereby the decrease in the gluconeogenic capacity of the liver is the result of an improvement of the insulin sensitivity caused by benfluorex. The primary effect could then for instance, be a reduction of counter hormones, like glucocorticoids, which would in turn improve the insulin sensitivity. The induction of expression of PEPCK by the corticosteroids will then diminish, resulting in the observed decrease in PEPCK. In this respect it is noteworthy that in our pilot study on young rats, that do not yet show a decreased insulin sensitivity, no effect of benfluorex on PEPCK was observed. In this case benfluorex decreased glycogen phosphorylase activity, indicating that the mechanism of action of benfluorex for these two enzymes is different.

It could be argued that the improvement of the insulin sensitivity might not be caused by a specific action of the drug, but by the observed weight loss of the treated animals. In other studies, however, it has been observed that benfluorex improves insulin sensitivity independent of weight loss [1].

In summary, our study showed that benfluorex decreases plasma insulin without changing the blood glucose concentration, thus probably improving the insulin sensitivity in aged rats. Determinations of hepatic enzyme activities revealed a decrease in PEPCK and glycogen phosphorylase, which is associated with an increase in hepatic glycogen content. Further work is necessary to determine whether the changes observed following benfluorex treatment are linked to a direct effect of the drug on the amount of the affected enzymes or the result of an indirect effect acting for instance via a drug-induced increase in insulin sensitivity. Finally, the specific reduction in both glycogen mobilization and gluconeogenesis in liver suggests that benfluorex could be of benefit in the treatment of insulin resistance and type II diabetes mellitus.

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REFERENCES

1. Arnaud O and Nathan C, Antiobesity and lipid-lowering agents with anti-diabetic activity. In: *New Anti-Diabetic Drugs* (Eds. Baily CJ and Flatt RP), pp. 133–142. Smith-Gordon, London, 1990.
2. Brindley DN, Hales P, Al-Sieni AII and Russell JC, Decreased serum lipids, serum insulin and triacylglycerol synthesis in adipose tissue of JCR:LA-corpulent rats treated with benfluorex. *Biochim Biophys Acta* **1085**: 119–125, 1991.
3. Geelen MJH, Mechanisms responsible for the inhibitory effects of benfluorex on hepatic intermediary metabolism. *Biochem Pharmacol* **32**: 1765–1772, 1983.
4. Geelen MJH and Wisserhof TA, Biochemical mechanisms of the inhibitory effects of benfluorex on hepatic intermediary metabolism. In: *Isolation, Characterization and Use of Hepatocytes* (Eds. Harris RA and Cornell NW), pp. 361–366. Elsevier Science Publishing Co., Amsterdam, 1983.
5. Goodman MN, Dluz SM, McElaney MA, Belur E and Ruderman NB, Glucose uptake and insulin sensitivity in rat muscle: changes during 3–96 weeks of age. *Am J Physiol* **244**: E93–E99, 1983.
6. Sinay IR, Arias P, Cagide A, Damilano S and Faingold MC, Additional metabolic effects of benfluorex in sulphonylurea-treated patient with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **32**: 541A, 1989.
7. Thomas JA, Schlender KK and Larner J, A rapid filter paper assay for UDPglucose-glycogen glycosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. *Anal Biochem* **25**: 486–499, 1968.
8. Villa-Moruzzi E, Locci-Cubeddu T and Bergamini E, An improved rapid assay of glycogen synthase. *Anal Biochem* **100**: 371–372, 1979.
9. Tan AWH and Nuttall FQ, Characteristics of the dephosphorylated form of phosphorylase purified from rat liver and measurement of its activity in crude liver preparations. *Biochim Biophys Acta* **410**: 45–60, 1975.
10. Ulm EH, Pogell BM, deMaine MM, Libby CB and Benkovic SJ, Fructose-1,6-diphosphatase from rabbit liver. In: *Methods in Enzymology* Vol. 42 (Ed. Wood WA), pp. 369–374. Academic Press, New York, 1975.
11. Fujii H and Miwa S, Pyruvate kinase. In: *Methods of Enzymatic Analysis* 3rd Edn, Vol. 3 (Ed. Bergmeyer HU), pp. 496–501. Verlag Chemie, Weinheim, 1983.
12. Bergmeyer HU, Grassl M and Walter H-E, Hexokinase. In: *Methods of Enzymatic Analysis* 3rd Edn, Vol. 2 (Ed. Bergmeyer HU), pp. 222–223. Verlag Chemie, Weinheim, 1983.
13. Vassault A, Lactate dehydrogenase. In: *Methods of Enzymatic Analysis* 3rd Edn, Vol. 3 (Ed. Bergmeyer HU), pp. 118–126. Verlag Chemie, Weinheim, 1983.
14. Karnovsky ML, Anchors JM and Zoccoli MA, Glucose-6-phosphatase from cerebrum. In: *Methods in Enzymology* Vol. 90 (Ed. Wood WA), pp. 396–402. Academic Press, New York, 1982.
15. Tielens AGM, van der Meer P, van den Heuvel JM and van den Bergh SG, The enigmatic presence of all gluconeogenic enzymes in *Schistosoma mansoni*. *Parasitology* **102**: 267–276, 1991.
16. Duff DA and Snell K, Limitations of commonly used spectrophotometric assay methods for phosphoenolpyruvate carboxykinase activity in crude extracts of muscle. *Biochem J* **206**: 147–152, 1982.
17. Ishikawa E, Ogushi S, Ishikawa T and Uyeda K, Activation of mammalian phosphofructokinase by ribose, 1,5-bisphosphate. *J Biol Chem* **265**: 18875–18878, 1990.
18. Bensadoun A and Weinstein D, Assay of proteins in the presence of interfering materials. *Anal Biochem* **70**: 241–250, 1976.
19. Devos P and Hers H-G, A molecular order in the synthesis and degradation of glycogen in the liver. *Eur J Biochem* **99**: 161–167, 1979.
20. Brindley DN, Bowley M, Burditt S, Pritchard PH, Lloyd-Davies KA and Boucrot P, The effect of administering *N*-(2-benzoyloxyethyl)norfenfluramine to rats on the hepatic synthesis of glycerolipids. *J Pharm Pharmacol* **28**: 676–682, 1976.

21. Duhault J, Boulanger M, Beregi L, Sicot N and Bouvier F, 780 SE: a new type of hypolipemic agent. Comparative assays in rats. *Atherosclerosis* **23**: 63–72, 1976.
22. Newgard CB, Hirsch LJ, Foster DW and McGarry JD, Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. A direct or an indirect pathway? *J Biol Chem* **258**: 8046–8052, 1983.
23. Storlien LH, Oakes ND, Pan DA, Kusunoki M and Jenkins AB, Syndromes of insulin resistance in the rat. Inducement by diet and amelioration with benfluorex. *Diabetes* **42**: 457–462, 1993.