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The regulation of gene expression by insulin is differentially impaired in the liver of the genetically obese-hyperglycemic Wistar fatty rat

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The regulation by insulin and carbohydrates of the gene expression of three key enzymes involved in glucose metabolism was studied in the liver of the Wistar fatty rat, a model of obese non-insulin-dependent diabetes mellitus. A high glucose or fructose diet, or insulin administration caused a similar magnitude of increase in the level of L-type pyruvate kinase mRNA in the liver of Wistar fatty rats and their lean littermates. However, the induction of glucokinase mRNA and repression of phosphoenolpyruvate carboxykinase mRNA by dietary glucose or insulin were impaired in the fatty rats, whereas fructose caused a similar decrease in phosphoenolpyruvate carboxykinase mRNA in both types of rats. These results indicate that the regulation of gene expression of glucokinase and phosphoenolpyruvate carboxykinase, but not of L-type pyruvate kinase, by insulin is impaired in the liver of the Wistar fatty rat.

L-Type pyruvate kinase; Glucokinase; Phosphoenolpyruvate carboxykinase; Non-insulin-dependent diabetes mellitus; Insulin

1. INTRODUCTION

Non-insulin-dependent diabetes mellitus (NIDDM) involves multiple defects in the liver, pancreatic B-cells and peripheral tissues such as muscle and adipose tissue. The Wistar fatty rat is a model of obese NIDDM [1]. This model was established by introducing the fa gene of the Zucker rat for obesity into the Wistar Kyoto strain, which is less sensitive to insulin and less tolerant to glucose than the Zucker rat. The fatty rats are obese, hyperphagic, hyperinsulinemic and hypertriglyceridemic. Males, but not females, develop hyperglycemia, glucouria and polyuria within 8 weeks of age. Previous studies have indicated that the activities of insulin-inducible enzymes such as L-type pyruvate kinase (LPK) and glucose-6-phosphate dehydrogenase in the liver of the fatty rats are higher than those in the lean rats [2]. However, the activity of glucokinase (GK), another insulin-inducible enzyme, was similar in the liver of both rats, and those of insulin-suppressible enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase were higher in the fatty, than the lean rats. Moreover, the activities of GK and glucose-6-phosphatase in the liver of the fatty rats do not change in response to an alteration in the plasma insulin level caused by fasting and refeeding with standard laboratory chow [2]. Thus, it is suggested that insulin resistance in the liver contributes to the development of hyperglycemia in Wistar fatty rats. However, the molecular mechanisms underlying these phenomena have not been elucidated.

We therefore investigated the effects of dietary glucose and insulin on LPK and GK gene expression in the liver of Wistar fatty rats. For comparison, we also examined the effect of dietary fructose on the expression of these genes, since it can induce a marked increase in LPK mRNA even in the liver of streptozotocin-induced diabetic rats [3–5]. In addition, regulation of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by these stimuli was examined since insulin regulation of this gene has been well characterized [6,7]. Here we report that the insulin regulation of the GK and PEPCK genes, but not that of LPK, is impaired in the liver of the Wistar fatty rat.

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Abbreviations: GK, glucokinase; LPK, L-type pyruvate kinase; PEPCK, phosphoenolpyruvate carboxykinase; NIDDM, non-insulin-dependent diabetes mellitus.

2. MATERIALS AND METHODS

2.1. *Animals*

Male Wistar fatty rats (fa/fa) and their lean littermates (Fa/?) were bred by mating heterozygous lean rats (Fa/fa) as reported previously [1]. They were maintained on laboratory chow and used at 8–10 weeks of age. The rats were starved for 48 h, then given a high carbohydrate diet containing 10% casein, 2% fat and 81% of either glucose or fructose (by weight) for the indicated periods. Rats were made insulin-deficient by intravenous injection of streptozotocin [3,8] and their blood was collected from the tail vein to determine plasma insulin and glucose levels 3 days after the injection. Rats with high glucose (>20

mM) and low insulin levels (<0.18 nM and <1.2 nM for the lean and fatty rats, respectively) were studied 7 days after streptozotocin administration. They were given a high glucose diet from 3 days before and throughout the experiment. Insulin was injected intraperitoneally at a dose of 0.2 units of rapid-acting insulin (Actrapid, Novo, Denmark) and subcutaneously, at a dose of 5 units of slow-acting insulin (Lente, Novo, Denmark) per 100 g body weight, respectively [5] The rats were killed at the indicated times after insulin injection and control rats were killed at zero time. Blood was collected and plasma was kept frozen at −20°C for plasma glucose and insulin determinations. Livers were immediately removed, frozen in dry ice and stored at −80°C for RNA isolation

2.2. *Isolation of RNA and Northern blotting*

Total cellular RNA was extracted from livers using acid guanidine–phenol–chloroform [9] The mRNAs for GK, LPK, PEPCK and β-actin were quantified by Northern blot hybridization using 20 μg of total RNA as described [5,10–12]. The integrity and amount of RNA applied were determined by ethidium bromide staining. The bands were analyzed by densitometry of autoradiograms as previously described [10,13]. The probes for GK, LPK and β-actin were as described previously [10,12] That for PEPCK was isolated from pPC116 [11], which was provided by Dr. D.K. Granner (Vanderbilt University, Nashville, TN, USA). They were labeled with [α-^{32}P]dATP (3,000 Ci/mmol) using random oligonucleotide primers [14].

2.3. *Analytical procedures*

Plasma glucose was measured enzymatically using Encore (Baker Instruments Co., PA, USA). Plasma insulin levels were determined by the double-antibody method using a commercial kit (Amersham International, UK).

3. RESULTS AND DISCUSSION

Wistar fatty and lean rats were starved for 48 h and then given a high glucose or fructose diet for 6 or 16 h. Control rats were killed at zero time. The levels of plasma insulin and hepatic mRNAs for LPK, GK, PEPCK and β-actin were determined. The plasma insulin level in the fatty rats was 0.98 ± 0.31, 2.4 ± 0.6 and 2.3 ± 0.5 nM in the zero time group, and those given glucose and fructose for 16 h, respectively, whereas that in the corresponding group of the lean rats was 0.08 ± 0.02, 0.58 ± 0.1 and 0.17 ± 0.1 nM, respectively. The LPK mRNA level was very low in the liver of both fatty and lean rats that had been starved for 48 h (Fig. 1A). Longer exposure to X-ray film revealed that the level of LPK mRNA was 2-fold higher in the fatty, than in the lean rats. The high glucose diet caused marked increases in the LPK mRNA level in the lean and fatty rats after 16 h. Although the induced mRNA level in the fatty rats was 1.8-fold higher than that in the lean rats, the extent of the mRNA induction was similar in both types of rats. This is consistent with the LPK activity in the liver of the fatty and lean rats reported previously [2]. Dietary fructose also stimulated expression of the LPK gene in both lean and fatty rats, and this response was faster than that of glucose, which agrees with previous results [4,5,13,15]. The extent of the LPK mRNA induction was similar in both types of rats given a fructose diet for 16 h.

On the other hand, an abnormal dietary response of GK mRNA was observed in Wistar fatty rats (Fig. 1B). The GK mRNA level in the liver of the starved fatty rats was about 24-fold higher than that in the starved lean rats. Giving them a glucose diet thereafter resulted in about a 100-fold increase in the level of GK mRNA in the lean rats, whereas only 2.1-fold increase was observed in Wistar fatty rats after 6 h. This induced GK mRNA level in the fatty rats was about 60% of that in the lean rats. These changes in the mRNA level can explain alterations in GK activity reported previously [2]. Dietary fructose also induced an increase in the GK mRNA levels in the lean rats, but to a much smaller extent (about 14-fold) than that by glucose. This diet did not affect GK gene expression in the fatty rats.

The repression of PEPCK gene expression by dietary glucose was also impaired in Wistar fatty rats (Fig. 1C). The starved level of PEPCK mRNA was not significantly different between both types of rats. Glucose feeding reduced PEPCK mRNA to less than 4 and less than 2% of the starved level in the lean rats after 6 and 16 h, respectively. In contrast, the mRNA level decreased to about 50 and 18% of the starved values in the fatty rats after 6 and 16 h, respectively. On the other hand, dietary fructose caused a similar decrease (about 60%) in PEPCK mRNA in both fatty and lean rats after 6 h. After 16 h, the reduction of this mRNA in the fatty rats was rather greater than that in the lean rats. These diets did not affect the level of β-actin mRNA in both types of rats (Fig. 1D).

Next, we examined the effect of exogenous insulin on gene expression in the liver of Wistar fatty and lean rats. Streptozotocin-treated rats were given a high glucose diet to prevent insulin-induced hypoglycemia. The results are shown in Fig. 2. The plasma insulin levels were markedly reduced in both rats by streptozotocin, but the values of the fatty rats (0.79 ± 0.18 nM) were still about 6-fold higher than those of the lean rats (0.13 ± 0.04 nM). In accordance with the insulin level, the LPK mRNA level in control fatty rats was 5-fold higher than that in control lean rats. However, the levels of GK and PEPCK mRNAs were not significantly different between the two types of control rats. Insulin caused about an 8.5- and 6-fold induction of LPK mRNA in the lean and fatty rats, respectively, after 16 h. This hormone also stimulated the GK gene expression about 25-fold in the lean rats after 6 h, but only 3.1-fold in the fatty rats after 6 h. The PEPCK gene expression in the lean rats was markedly reduced by insulin after 6 and 16 h, whereas the extent of the decrease was much less in the fatty rats. Insulin did not cause a change in the β-actin mRNA level in both types of rats. These results were consistent with those obtained using the glucose-fed normal fatty and lean rats described above.

The present results indicate that the regulation of the gene expression of GK and PEPCK, but not of LPK by dietary glucose or insulin, is impaired in the Wistar fatty

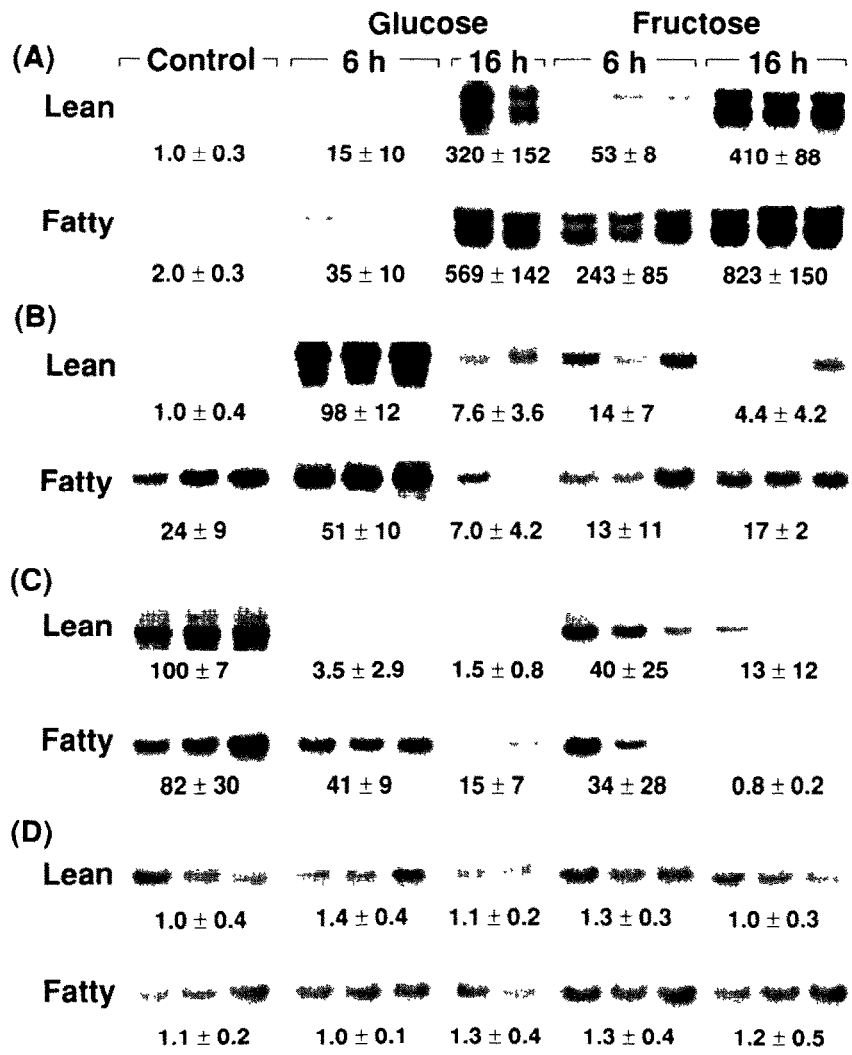

Fig. 1. The effects of dietary glucose and fructose on the levels of LPK (A), GK (B), PEPCK (C) and β-actin (D) mRNAs in the liver of Wistar fatty and lean rats The rats were given a high glucose or fructose diet for the indicated periods after starvation for 48 h. Control rats were killed at zero time. Total RNA was isolated from individual livers and analyzed by Northern blot hybridization. Values below the autoradiograms are means ± S.D. and are normalized to the value of control lean rats.

rat. The reduced response of GK mRNA to dietary fructose compared with glucose can be explained by their different abilities to stimulate insulin secretion [15,16]. Dietary fructose cannot induce GK mRNA in streptozotocin-induced diabetic rats [17]. However, this may not be so for PEPCK. The reduction of PEPCK mRNA caused by dietary fructose in the fatty rats was similar to or even greater than that in the lean rats. Although little is known about the regulatory mechanism of PEPCK gene expression by fructose, this cannot be explained by insulin secretion. We assume that fructose affects PEPCK gene expression by a mechanism independent of insulin such as that of LPK [4,5,13].

It has been reported that starvation causes increased GK activity in fatty rats, but decreases the level in lean rats [2]. In agreement with this, the GK mRNA level in the liver of the starved fatty rats was 24-fold higher than that in the lean rats. In the insulin deficient state caused by streptozotocin, however, both types of rats had a similar GK mRNA level. This discrepancy remains to be solved.

The mechanism of insulin resistance in the Wistar fatty rat remains to be elucidated. Whatever the mechanism is, it should explain the differential impairment of insulin action in this rat. Evidence suggests that the insulin receptor is not the major contributor. Although insulin binding to the plasma membrane is reportedly reduced in Wistar fatty rats, a similar decrease is also observed in lean rats with the fa gene [18]. About 67% of the lean rats used in this study are presumed to have the fa gene. Moreover, the tyrosine kinase activity of the insulin receptor is reportedly somewhat higher in the liver of Wistar fatty, than in lean rats [19]. We did not determine the transcription rates of the genes in the fatty and lean rats. Insulin is reported to predominantly regulate the transcription of the three genes examined

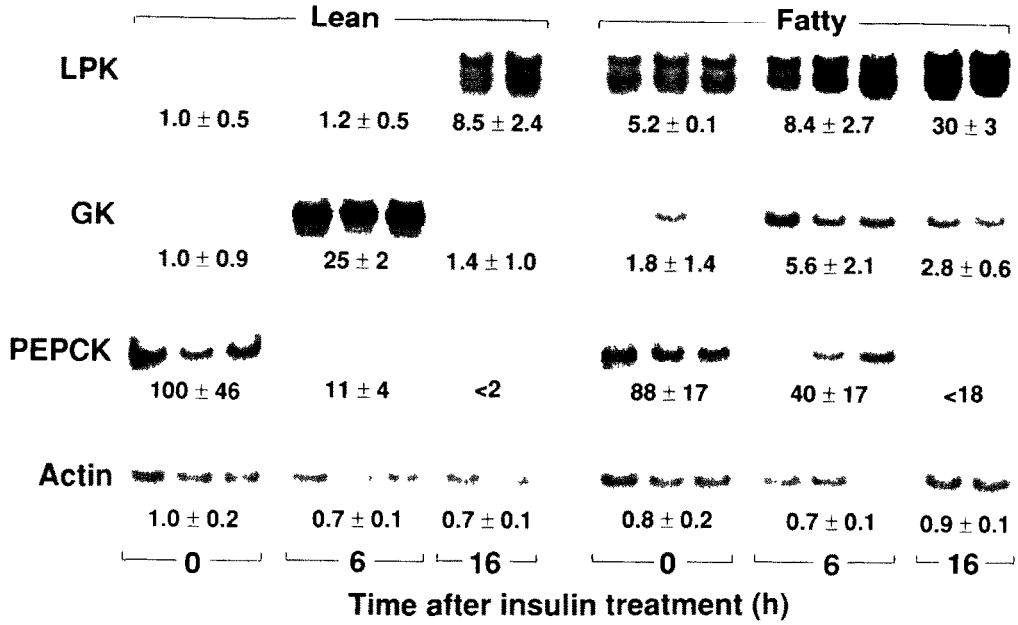

Fig. 2 The effect of insulin on the levels of LPK, GK, PEPCK, and β-actin mRNAs in the liver of Wistar fatty and lean rats. Streptozotocin-treated rats were injected with insulin Total RNA was isolated from individual livers at the indicated times and analyzed by Northern blot hybridization. Values below the autoradiograms are means ± S D. and are normalized to the value of control lean rats.

in this study, but their mechanisms appear to be different [6,7,17]. We therefore presume that the impaired expression of the GK and PEPCK genes occurred at the transcriptional level. Thus, we speculate that the signaling pathway from the insulin receptor to the GK and PEPCK genes, but not to that of LPK, is defective at a point beyond the receptor kinase in the fatty rats. Further studies are required to resolve this issue.

It has been postulated that a glucose metabolite is an active inducer of LPK [20]. In line with this, we demonstrated that the stimulation of LPK gene expression by insulin requires glucose metabolism [10] and that the same *cis*-acting element of the LPK gene was responsible for transcriptional stimulation by dietary fructose as well as glucose [21]. It has been postulated that the metabolite is generated as a consequence of the stimulation of GK gene transcription by insulin [7]. If so, the reduced stimulation of the GK gene by insulin should also impair the regulation of LPK gene expression. Thus, the present results do not support this hypothesis and another enzyme may be considered as the prime target for insulin action on LPK.

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