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# RAPID EFFECTS OF INSULIN AND GLUCOSE ON THE HEPATIC INCORPORATION OF GLUCONEOGENIC SUBSTRATES INTO GLYCERIDE GLYCEROL AND GLYCOGEN

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**Abstract**—1. The hepatic utilization of gluconeogenic substrates was investigated shortly after portal infusion of either insulin or glucose in fasted rats.

2. After 20 min of insulin infusion blood glucose concentration decreased. However, neither glucose generation from precursors such as alanine or pyruvate nor their incorporation into fatty acids was modified. Under these conditions, insulin rapidly increased the incorporation of gluconeogenic substrates into the hepatic glyceride glycerol fraction. Insulin treatment led to a decrease in substrate incorporation into liver glycogen.

3. After 20 min of portal glucose infusion both plasma insulin and glucose concentrations increased and the incorporation of pyruvate into hepatic glyceride glycerol and into glycogen was also stimulated.

4. A close relationship was observed between blood glucose concentrations and the level of incorporation of gluconeogenic substrates into liver glycogen.

5. In conclusion, during fasting insulin stimulates the incorporation of gluconeogenic substrates into the glycerol moiety of hepatic glycerides, which may be the preferential mechanism through which fatty acid esterification is accomplished during refeeding. This effect of insulin is rapid and detected even before other classical modifications induced by the hormone such as gluconeogenesis inhibition or lipogenesis activation. Furthermore, the effect is not related to insulin-induced hypoglycemia since glucose infusion mimics insulin action on glyceride glycerol synthesis.

## INTRODUCTION

Insulin is known to promote a complex array of metabolic modifications in liver that include activation of lipogenesis, fatty acid esterification and stimulation of glycogen synthase activity (Bloch and Kramer, 1948; Topping and Mayes, 1972; Geelen *et al.*, 1978; Ciudad *et al.*, 1981), as well as inhibition of fatty acid oxidation, ketogenesis and hormonally-induced gluconeogenesis (Topping and Mayes, 1972; Harano *et al.*, 1972; Claus and Pilkis, 1976; Feliu *et al.*, 1976; Laker and Mayes, 1984).

During fasting hepatic gluconeogenesis is fully activated and plays a central metabolic role; it generates not only the glucose required to maintain its homeostasis, but it also provides precursors for the synthesis of glycogen and glyceride glycerol (Soley *et al.*, 1983; Zorzano and Herrera, 1988). In fact, during fasting glyceride synthesis requires alpha-glycerol phosphate from gluconeogenic origin (Zorzano and Herrera, 1988). Provided that insulin regulates fatty acid esterification as well as gluconeogenesis and glycogen synthase in liver, it was of interest to explore a possible short-term regulatory action of insulin on the gluconeogenic synthesis of glycogen as well as the glycerol moiety of hepatic glycerides.

Thus, the present investigation was designed to present evidence of a direct and rapid effect of insulin on the channelling of gluconeogenic intermediates at the level of dihydroxyacetone phosphate in hepatic glyceride generation and at the level of glucose-6-phosphate in glycogen synthesis. To that effect, insulin was infused *in vivo* through the portal vein and its metabolic effects on hepatic utilization of gluconeogenic substrates were examined. Furthermore, and in order to assess whether the observed effects were attributable to insulin *per se* or to insulin-induced hypoglycemia, glucose was also infused through the portal vein and its effects on the utilization of gluconeogenic substrates investigated.

## MATERIALS AND METHODS

Fed and 24 hr fasted female Sprague–Dawley or male Wistar rats were used throughout this study. On the experimental day, rats were anesthesized by i.v. injection of 33 mg/kg body weight of sodium pentobarbital and were subjected to the surgical procedure previously described (Soley *et al.*, 1983; Zorzano and Herrera, 1986). Briefly, it consisted in introducing a catheter through the ileocolic vein up to the level of the portal vein. After suturing the abdominal wall, another cannula was introduced through the right jugular vein passing by the superior cava vein down to the level of the suprahepatic veins. Once surgery was finished, a saline–albumin solution (0.5 mg bovine albumin/ml of 0.9% NaCl) was infused through the cannula in

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the portal vein, at a rate of 5 ml/hr for 10 min, after which a pulse of radioactive substrate was administered. In female Sprague–Dawley rats, 10 $\mu$Ci and 0.2 mmol of either [U-$^{14}$C]alanine or [3-$^{14}$C]pyruvate were injected. In male Wistar rats, 2 $\mu$Ci and 0.2 mmol of [3-$^{14}$C]pyruvate was administered. The administration of the substrate was followed by an infusion with either saline–albumin (control group), insulin at a rate of 33 mU/min or glucose at a rate of 43 or 116 $\mu$mol/min. Infusions were always performed at a rate of 5 ml of the corresponding solution per hour. Blood samples (150 $\mu$l) were collected from the catheter placed in the suprahepatic veins at different times after the tracer. After 20 min, an aliquot of liver was rapidly excised and frozen in liquid nitrogen. Blood aliquots were deproteinized with 5% perchloric acid and neutralized supernatants used for glucose and alanine determinations (Zorzano and Herrera, 1986) and for the purification of [$^{14}$C]glucose, [$^{14}$C]alanine or [$^{14}$C]pyruvate by ion-exchange chromatography (Zorzano *et al.*, 1986). Plasma insulin was determined by radioimmunoassay specific for rat (kindly donated by Novo Industri A/S, Denmark). One aliquot of frozen liver was digested with 30% KOH for glycogen purification (Good *et al.*, 1933) and radioactivity and glucose concentration were quantified after acid hydrolysis. Another aliquot of frozen liver was used for lipid extraction (Folch *et al.*, 1957) and the extracts were fractionated for fatty acid purification using the method of Kerpel *et al.* (1961). All radioactive values were adjusted to an initial value of $1 \times 10^6$ dpm of administered tracer. Statistical analysis was by Student's *t* test.

## RESULTS

In order to investigate the effect of insulin on *in vivo* hepatic utilization of gluconeogenic substrates, in an initial set of experiments, the hormone was infused through the portal vein of 24 hr fasted female Sprague–Dawley rats at a rate of 33 mU/min. This rate of infusion has been previously used in our laboratory (Soley *et al.*, 1985b) and raises plasma insulin levels higher than 300 $\mu$U/ml after 5 min of its infusion. As a result of insulin infusion, blood glucose concentration markedly decreased 10 min after the initiation of the experiment (data not shown), evidencing insulin-stimulated peripheral glucose utilization. On the other hand, the hormone did not alter the alanine disappearance rate (data not shown). Results of hepatic glucose synthesis from alanine and pyruvate obtained in the same rats are shown in Table 1. The specific activity of glucose appearing in blood from the injected alanine, increased progressively with time reaching a plateau after 10 min of administration. When pyruvate was the substrate administered, specific activity of glucose reached a maximum after 5 min of its injection. In keeping with previous observations from our laboratory (Zorzano and Herrera, 1986), glucose synthesis from pyruvate was more efficient than from alanine and the rate of gluconeogenesis in the fasted group was considerably higher than in the fed group (results not shown). Under these conditions, insulin had no effect on gluconeogenesis from either alanine or pyruvate at all the times investigated (Table 1). After 20 min of substrate administration, radiolabeled alanine and pyruvate were also incorporated into liver glycogen and lipids in fasted female Sprague–Dawley rats (Table 2). This is not surprising since it has been previously reported that during fasting, glycogen as well as glyceride glycerol are synthesized as a consequence of gluconeogenic activity (Soley *et al.*, 1983; Zorzano and Herrera, 1988). In the fasted control group, incorporation of the radioactive precursors, alanine and pyruvate, into glycogen and glyceride glycerol, was proportional to the attained specific activity of glucose (see Table 1). Incorporation into liver fatty acids was minimal and insulin did not modify the lipogenic pathway. Under these conditions, insulin infusion markedly increased the incorporation of gluconeogenic substrates into hepatic glyceride glycerol fraction, both from alanine and pyruvate. Incorporation of gluconeogenic precursors into hepatic glycogen was somewhat but not significantly diminished by insulin infusion (Table 2).

In order to test whether our findings were sex- or strain-dependent, another set of experiments was carried out in fasted male Wistar rats that were administered [3-$^{14}$C]pyruvate and subsequently infused with saline, insulin or glucose. After 20 min of substrate administration, infusion of insulin alone caused a decrease in blood glucose concentration

Table 1. Glucose synthesis from alanine and pyruvate in control and insulin-infused rats

| Min after the tracer | Glucose sp. act. (dpm/$\mu$mol) | |
|---|---|---|
| | Control | Insulin |
| *Alanine* | | |
| 2 | 132 ± 14 | 152 ± 23 |
| 5 | 251 ± 41 | 305 ± 40 |
| 10 | 316 ± 32 | 341 ± 22 |
| 20 | 349 ± 30 | 408 ± 67 |
| *Pyruvate* | | |
| 2 | 504 ± 60 | 533 ± 107 |
| 5 | 701 ± 76 | 676 ± 65 |
| 10 | 660 ± 76 | 636 ± 81 |
| 20 | 616 ± 71 | 623 ± 38 |

Values are means ± SE of 6–7 observations/group. Fasted female Sprague–Dawley rats received through the portal vein a pulse of [U-$^{14}$C]alanine or [3-$^{14}$C]pyruvate and a subsequent infusion of saline or insulin (33 mU/min). Blood samples were collected from the cava vein at the level of the suprahepatic vein at different times after the administration of the substrate, and specific activity of glucose was determined. All radioactive values were adjusted to an initial value of $1 \times 10^6$ dpm of administered tracer. Differences between control and insulin-infused groups were insignificant.

Table 2. Incorporation of [U-$^{14}$C]alanine and [3-$^{14}$C]pyruvate into hepatic lipids and glycogen in control and insulin-infused rats

| | [$^{14}$C]Glyceride glycerol (dpm/g tissue) | $^{14}$C-labelled fatty acids (dpm/g tissue) | [$^{14}$C]Glycogen (dpm/g tissue) |
|---|---|---|---|
| *Alanine* | | | |
| Control | 399 ± 61 | 32 ± 9 | 286 ± 188 |
| Insulin | 866 ± 36* | 30 ± 2 | 214 ± 42 |
| *Pyruvate* | | | |
| Control | 623 ± 78 | 42 ± 4 | 735 ± 242 |
| Insulin | 941 ± 90* | 36 ± 3 | 476 ± 112 |

Values are means ± SE of 6–7 observations/group. Fasted female Sprague–Dawley rats received a pulse of [U-$^{14}$C]alanine or [3-$^{14}$C]pyruvate through the portal vein after which they were infused with saline or insulin (33 mU/min). Livers were frozen after 20 min of the administration of substrates, for purification of glycogen and different fractions of lipids. All radioactive values were adjusted to an initial value of $1 \times 10^6$ dpm of administered tracer. *Indicates a value significantly different from that of control group at $P < 0.05$.

Table 3. Effect of insulin or glucose infusion on the incorporation of [3-$^{14}$C]pyruvate into hepatic lipids and glycogen

| | [$^{14}$C]Glyceride glycerol (dpm/g tissue) | $^{14}$C-labelled fatty acids (dpm/g tissue) | [$^{14}$C]Glycogen (dpm/g tissue) |
|---|---|---|---|
| Control | 1139 ± 176 | 45 ± 9 | 512 ± 100 |
| Insulin | 1607 ± 97* | 76 ± 22 | 183 ± 24* |
| Glucose | 1664 ± 98* | 61 ± 11 | 2977 ± 411* |

Values are means ± SE of 8 observations per group. Fasted male Wistar rats received a pulse of 2 μCi/0.2 mmol of [3-$^{14}$C]pyruvate and immediately after an infusion with saline, insulin (33 mU/min) or glucose (116 μmol/min) through the portal vein. Livers were frozen after 20 min of substrate administration, for purification of glycogen and different fractions of lipids. All radioactive values were adjusted to an initial value of $1 \times 10^6$ dpm of administered tracer. *Indicates a value significantly different from that of control group at $P < 0.05$.

(2.3 ± 0.3 mM) as compared to the control group (4.2 ± 0.3 mM), whereas glucose infusion gave rise to high blood glucose levels (20.2 ± 1.3 mM). Five minutes after the start of the experiment, plasma insulin levels markedly increased by insulin (357 ± 22 μU/ml) or glucose (174 ± 12 μU/ml) as compared to the control group (70 ± 14 μU/ml). Under these conditions both insulin or glucose infusion increased the incorporation of [3-$^{14}$C]pyruvate into the hepatic glyceride glycerol fraction, with no modification of the lipogenic pathway (Table 3). Insulin caused a decrease in the incorporation of pyruvate into liver glycogen, whereas the infusion of glucose caused a tremendous increase in glycogen synthesis (Table 3) in keeping with previous observations from our laboratory (Soley *et al.*, 1985a).

As a matter of fact, it was possible to detect a close relationship between blood glucose concentrations attained at the end of the experiment and the incorporation of the administered radioactive pyruvate into liver glycogen when plotting the means from all the experimental groups (Fig. 1). Thus, glucose seems to be an important short-term modulator for the

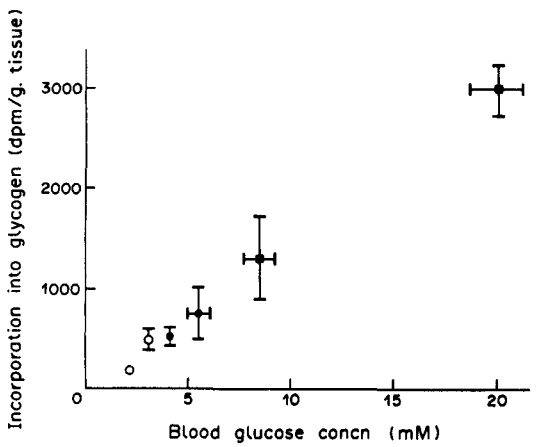


Fig. 1. Relationship between blood glucose concentration and incorporation of [3-$^{14}$C]pyruvate into hepatic glycogen after 20 min of portal infusion of insulin or glucose in the 24 hr fasted rat. Points are means ± SE for 6–8 rats. Control groups (●); insulin-infused groups (33 mU/min) (○); glucose-infused groups (43 or 116 μmol/min) (■).

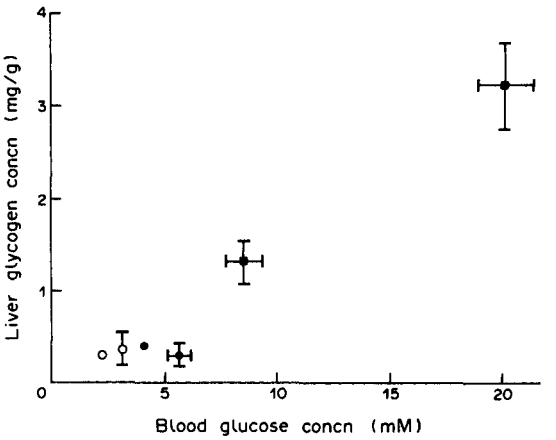


Fig. 2. Relationship between blood glucose concentration and liver glycogen concentration after 20 min of portal infusion of insulin or glucose in the 24 hr fasted rat. Points are means ± SE for 6–8 rats. Control groups (●); insulin-infused groups (33 mU/min) (○); glucose-infused groups (43 or 116 μmol/min) (■).

incorporation of gluconeogenic metabolites into liver glycogen. Furthermore, there is a relationship between blood glucose concentrations observed at the end of the experiment and the glycogen concentration attained by the liver of fasted rats after various treatments (Fig. 2). However, we failed to demonstrate a relationship between plasma insulin concentrations and the incorporation of pyruvate into liver glycogen (data not shown).

## DISCUSSION

Our data indicate that insulin causes an augmented hepatic channeling of gluconeogenic substrates at the level of dihydroxyacetone phosphate that are directed to the esterification of fatty acids. Insulin effect is rapid, and in fact, an increase in the radioactivity incorporated into the hepatic glycerol glyceride fraction was observed after only 20 min of insulin infusion. Our results are in keeping with previous reports of insulin-enhanced fatty acid esterification (Topping and Mayes, 1972) detected after 2 hr of perfusion in the presence of insulin. Triacylglycerides and phospholipids are the quantitatively most important glycerol-containing lipids synthesized in liver (Vavrecka *et al.*, 1969; Topping and Mayes, 1972). Given that insulin has been reported to not modify phospholipid metabolism in isolated hepatocytes (Sakai and Wells, 1986), the labeled glycerides appearing in our experiments are most likely to be triacylglycerides. Insulin action on glyceride synthesis might be caused by an increase in the gluconeogenic availability of alpha-glycerol phosphate, a limiting factor in the esterification of fatty acids in liver (Debeer *et al.*, 1981; Declercq *et al.*, 1982), perhaps mediated by modification of the redox state. Alternatively, insulin action might involve modulation of regulatory enzymes of the esterification pathway. In that regard, it has been reported that insulin increases the activity of mitochondrial glycerol phosphate acyltransferase within 30 min after its addition to the perfused liver (Bates

*et al.*, 1977). Insulin effect stimulating the incorporation of pyruvate into hepatic glyceride glycerol is not mediated via a decrease in blood glucose concentration and, in fact, insulin action on glyceride glycerol synthesis was mimiced by portal glucose infusion.

It is well established that insulin inhibits hepatic gluconeogenesis, specially the stimulation induced by glucagon or cAMP (Friedmann *et al.*, 1967; Sladek and Snarr, 1974; Claus and Pilkis, 1976; Feliu *et al.*, 1976). The mechanisms through which insulin acts involve a decrease in the level of expression of the gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase or glucose-6-phosphatase (Weber *et al.*, 1965) as well as a rapid activation of pyruvate kinase (Feliu *et al.*, 1976; Claus *et al.*, 1979). However, this pattern is difficult to reconcile with results obtained during refeeding, a condition in which plasma insulin concentrations are elevated. Recently reported data have shown that during refeeding, liver glycogen synthesis is greatly enhanced by a process dependent upon gluconeogenic activity (Newgard *et al.*, 1983, 1984). Our data suggest that the inhibitory action of insulin on hepatic gluconeogenesis must take longer than 20 min to display itself *in vivo*. In addition, they support the concept that an active gluconeogenic pathway might be maintained during the transition from fasted to fed state. Therefore, they allow to explain the indirect pathway of glycogen synthesis, via 3-carbon precursors.

Insulin and glucose infusion through the portal vein rapidly modified the gluconeogenic substrate incorporation into liver glycogen; thus, insulin decreased whereas glucose increased the percentage of the pyruvate administered incorporated in glycogen. Furthermore, there was a close relationship between the level of incorporation of pyruvate into liver glycogen and the blood glucose concentration attained at the end of the infusion in the various experimental groups. These results suggest that gluconeogenic synthesis of liver glycogen is exquisitely sensitive to rapid changes in blood glucose concentration (in cell?) and probably less dependent on insulin as a short term modulator. This is in keeping with previous reports obtained using the euglycemic–hyperinsulinemic clamp technique in the rat, where it was found that in the presence of normoglycemia, insulin only moderately stimulates glycogen synthase activity and glycogen deposition in liver (Kruszynska *et al.*, 1986). In that regard, glucose might be a critical short-term modulator of liver glycogen synthesis by activating glycogen synthase phosphatase (Gilboe and Nuttall, 1984). In addition, we have found that an increase in blood glucose concentration, provoked by portal glucose infusion, leads to an increase in liver glycogen concentration, reflecting a stimulated net glycogen synthesis. Under those conditions, we did not measure the contribution of the direct glucose → glucose-6-phosphate → glycogen) vs indirect (gluconeogenic) pathways for liver glycogen synthesis. However, according to Lang *et al.* (1986), at high circulating glucose concentrations (> 15 mM) hepatic glycogen repletion appears to proceed primarily via the direct pathway.

In conclusion, the results obtained herein demonstrate that, in fasted rats, insulin stimulates the esterification of fatty acids to the alpha-glycerol phosphate backbone, synthesized from gluconeogenesis. This effect of insulin is rapid and takes place when the only action of the hormone is the activation of peripheral glucose utilization and no effects of insulin inhibiting gluconeogenesis or activating lipogenesis were yet apparent. In addition, this effect of the hormone is not mediated by the insulin-induced hypoglycemia and is mimic by glucose infusion. Furthermore, the data support the contention that the stimulated gluconeogenic flux of fasting is maintained early during refeeding, in spite of high levels of plasma insulin present in that situation. Incorporation of gluconeogenic precursors into liver glycogen is also subjected to rapid modulation but this seems to be highly dependent on the blood glucose concentration and independent of circulating insulin.

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