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Short-term control of hepatic lipogenesis by insulin

Anton C. Beynen, Math J. H. Geelen and Simon G. Van den Bergh

This article presents evidence that insulin acutely affects carbohydrate and lipid metabolism in isolated rat hepatocytes. A coherent picture emerges of the concerted mechanism by which insulin sets the stage for lipogenesis in the hepatocyte.

The liver of an animal ingesting a high-carbohydrate diet must take up the glucose in the blood, store some as glycogen and transform the rest into fatty acids. If starved rodents are re-fed a glucose-rich meal, the liver may contribute as much as 50% to the fatty acid synthesis of the body[1,2]. This increase in lipogenesis upon refeeding is more pronounced and prompter in the liver than in any other tissue[1], thus emphasizing the fact that the liver is of considerable importance as a lipogenic organ. The fatty acids which are synthesized *de novo* in the liver may either be stored in the liver as triacylglycerols or released into the blood stream as constituents of lipoproteins.

Re-feeding induces in the liver the synthesis of a number of lipogenic enzymes, but it takes several hours before this results in an increase in the amount of active enzyme molecules[3]. The rate of fatty acid synthesis is, however, greatly increased well before this long-term regulatory mechanism is effective. Here we have restricted our attention to the short-term control of hepatic lipogenesis.

The acute acceleration of hepatic fatty acid synthesis observed in rodents after ingestion of glucose-rich meals[1] could be explained by an increased availability of the substrate, glucose in the portal blood[4]. This is supported by our observation that supplementing isolated hepatocytes with glucose markedly enhances fatty acid synthesis as measured by the incorporation of label from tritiated water (Table I).

Like the blood level of glucose[4], that of insulin increases dramatically and promptly (within 30 min) during the switch from starvation to refeeding[6]. It has been stated repeatedly that insulin, when present as the sole hormone, has no acute effect on hepatic lipogenesis[7], glycogen metabolism[8] and gluconeogenesis[9]. Addition of insulin to hepatocytes in suspension, however, stimulates fatty acid synthesis within 30 min[5,10] (see also Table I). Here we present the evidence that insulin rapidly affects carbohydrate and lipid metabolism in isolated rat hepatocytes. A concerted mechanism is described by which insulin acutely stimulates the conversion of glucose into fatty acids by the liver. Fig. 1 depicts the pathway of the hepatic synthesis of triacylglycerol from glucose.

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Glycolysis

Insulin increases the flux through glycolysis in hepatocytes prepared from meal-fed rats as judged by the hormone-induced elevation of the sum of lactate and pyruvate concentrations[5]. In addition, insulin was found to stimulate the incorporation of D-[U-^{14}C]glucose into lactate (Table II).

Potential regulatory sites within the glycolytic sequence are considered to be phosphofructokinase and pyruvate kinase. Insulin rapidly counteracts glucagon-mediated inhibition of phosphofructokinase in isolated hepatocytes, but is ineffective if added as the sole hormone[11]. In hepatocytes, insulin decreases the concentration of fructose 1,6-biphosphate[12] which is not compatible with activation of phosphofructokinase by insulin. Possibly the

TABLE I
Effects of glucose and insulin on fatty acid synthesis by isolated hepatocytes. Hepatocytes were isolated from meal-fed rats and incubated for 1 h with ^3H_2O (0.3 mCi/ml) exactly as previously described[5].

Additions	Fatty acid synthesis (nmol acetyl units incorp. h^{-1} mg protein^{-1})
None	16
Glucose (10 mM)	31
Glucose + insulin (85 nM)	45

stimulation of pyruvate kinase by insulin, which has been demonstrated in the perfused rat liver[13], is responsible for this. Elevation of glycerol 3-phosphate levels in hepatocytes exposed to insulin[14] may be the result of an increased cytoplasmic (NADH)/(NAD^+) ratio, which is caused by addition of insulin (Table II).

TABLE II

Effect of insulin on glycolysis, glycogenesis and gluconeogenesis. Hepatocytes were isolated from meal-fed rats and incubated for 1 h with 10 mM D-[U-^{14}C]glucose (0.04 Ci/mol) or (last line) with unlabelled glucose and 1 mM [3-^{14}C]pyruvate (0.045 Ci/mol), as described previously[5,10].

	Control	Insulin (85 nM)
Glucose incorporation into lactate (nmol h^{-1} mg protein^{-1})	101 ± 1	120 ± 5[b]
Lactate accumulation (mM)	1.86 ± 0.12	2.02 ± 0.05[b]
Pyruvate accumulation (mM)	0.36 ± 0.02	0.31 ± 0.01[b]
Lactate/pyruvate ratio	5.17	6.52
Glucose incorporation into glycogen (nmol h^{-1} mg protein^{-1})	17 ± 3	34 ± 6[a]
Pyruvate incorporation into glucose (nmol h^{-1} mg protein^{-1})	27 ± 2	18 ± 3[a]

Each figure represents the mean ± SD of three different incubations. Statistical analysis was performed according to *Student's t* test. Versus control; [a], $p < 0.01$; [b], $p < 0.02$.

Mitochondrial function

Insulin activates pyruvate kinase[13] but slightly, and significantly, lowers pyruvate concentrations in hepatocyte suspensions (Table II). This would suggest that activation of pyruvate kinase is not the overall rate-limiting step of the pathway of fatty acid synthesis in the liver. One could explain this fall in pyruvate concentration by the activation of pyruvate dehydrogenase in mitochondria which, as we have shown, is increased upon treatment of the intact hepatocytes with insulin[15]. Furthermore, in mitochondria from insulin-treated hepatocytes the oxidation of succinate is depressed[15]. Slowing down the Krebs cycle would lead to the formation and efflux of citrate from the mitochondria, which in turn would favor fatty acid synthesis, since substrate availability would be increased.

Fatty acid synthesis *per se*

In fact, however, insulin slightly lowers citrate concentrations in hepatocytes prepared from meal-fed rats[10,16]. This points to an insulin-induced stimulation of a step in fatty acid synthesis beyond the formation of cytosolic citrate. This conclusion is also supported by the observation that insulin stimulates the incorporation of [1-^{14}C]-acetate into fatty acids, an effect which cannot be explained by a hormone-mediated alteration of the specific radioactivity of the cytosolic acetyl-CoA precursor pool[10,17]. Furthermore, acetyl-CoA carboxylase, generally considered to be the rate-limiting enzyme in fatty acid biosynthesis, was indeed found to be subject to short-term endocrine control. Insulin rapidly stimulates acetyl-CoA carboxylase activity as measured in homogenates of hormone-treated rat hepatocytes[5,10]. Our results concerning insulin effects both on fatty acid synthesis (as determined by the ^3H_2O procedure) and on acetyl-CoA carboxylase activity were recently confirmed by Witters and co-workers[18]. Upon a brief exposure to insulin, isolated hepatocytes display an increased cellular content of malonyl-CoA[10], the product of acetyl-CoA carboxylation. This substantiates the role of acetyl-CoA carboxylase as a target in the short-term control of fatty acid synthesis by insulin. We propose that the rate of fatty acid biosynthesis is determined by the level of malonyl-CoA, which sets the velocity of the reaction catalysed by fatty acid synthase[10].

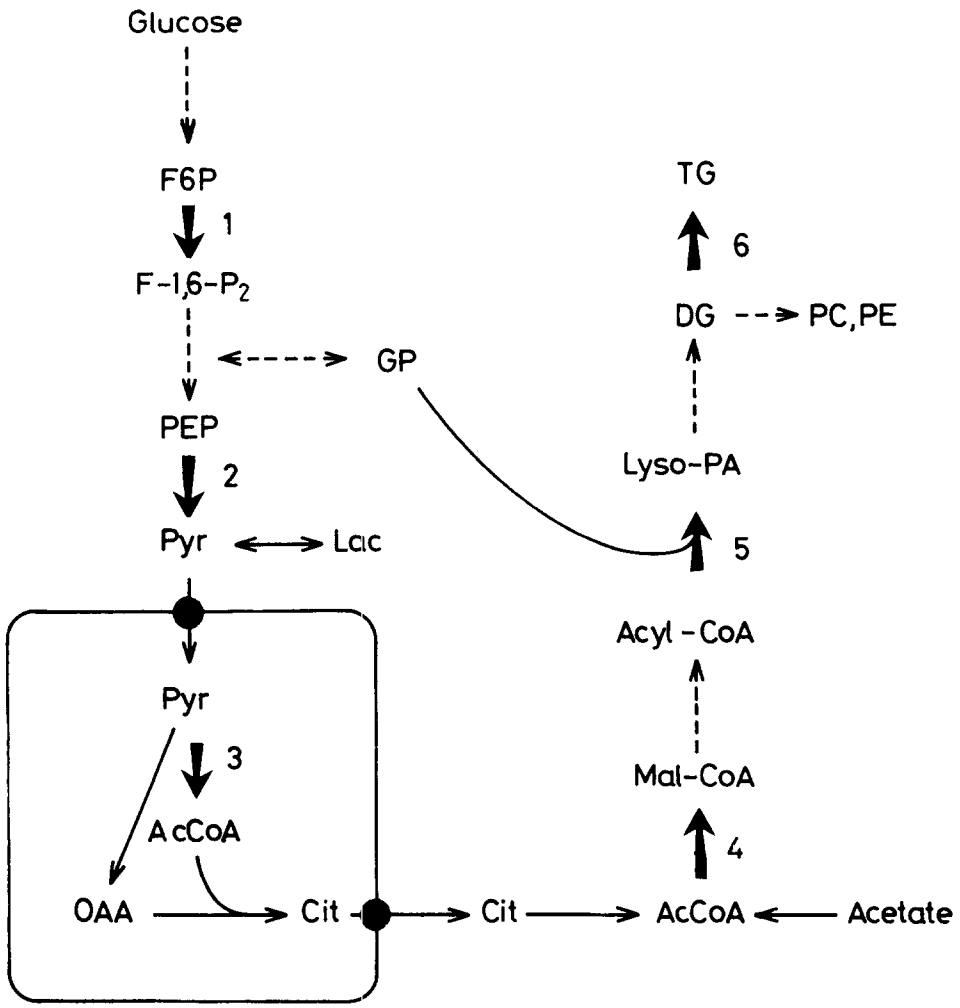

Fig. 1. Enzymatic sites of acute control of hepatic triacylglycerol synthesis. F6P, fructose 6-phosphate; F-1,6-P$_2$, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvate; Pyr, pyruvate; Lac, lactate; AcCoA, acetyl-CoA; OAA, oxaloacetate; Cit, citrate; Mal-CoA, malonyl-CoA; Lyso-PA, lysophosphatidate; DG, diacylglycerol; TG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; GP, glycerol 3-phosphate. The regulatory enzymes: 1, phosphofructokinase (EC 2.7.1.11); 2, pyruvate kinase (EC 1.7.1.40); 3, pyruvate dehydrogenase (EC 1.2.4.1); 4, acetyl-CoA carboxylase (EC 6.4.1.2); 5, glycerolphosphate acyltransferase (EC 2.3.1.15); 6, diacylglycerol acyltransferase (EC 2.3.1.20).

Triacylglycerol synthesis

Since long-chain acyl-CoA esters are potent inhibitors of both pyruvate dehydrogenase and acetyl-CoA carboxylase, lipogenesis is dependent upon the liver's capacity to dispose of these CoA esters. Insulin stimulates fatty acid esterification in short-term incubations of rat hepatocytes[14], thereby preventing feedback inhibition of lipogenesis by long-chain acyl-CoA. The stimulation of fatty acid esterification by insulin may be due to a combination of an activation of glycerol-phosphate acyltransferase, as demonstrated in the perfused liver[19], and an elev-

ation of the intracellular concentration of glycerol 3-phosphate[14], the precursor for the glycerol backbone of the glycerolipids.

The microsome-bound diacylglycerol acyltransferase, the only enzyme in the pathway exclusively concerned with triacylglycerol synthesis, was recently found to be inhibited by glucagon[20]. Whether insulin activates this enzyme remains to be established.

Interconversion of regulatory enzymes between active and inactive forms by phosphorylation–dephosphorylation cycles

Studies with partially purified enzyme systems have demonstrated that the six regulatory enzymes involved in the conversion of glucose into triacylglycerols are subject to short-term regulation by covalent modulation (for review, see Ref. 21). It should be noted that in the case of glycerolphosphate acyltransferase and diacylglycerol acyltransferase there is only suggestive evidence for regulation of enzyme activity by a phosphorylation–dephosphorylation mechanism. All enzymes are active in the dephosphorylated mode and inactive in their phosphorylated state. Immediate changes in enzyme activity *in vitro* can be evoked by phosphorylation and dephosphorylation by protein kinases and phosphoprotein phosphatases, respectively.

For some enzymes evidence has been presented that within the intact cell, phosphorylation–dephosphorylation cycles are also involved in the endocrine control of their activity. Glucagon causes phosphorylation of hepatic phosphofructokinase[22], pyruvate kinase[23] and acetyl-CoA carboxylase[16,24], which is accompanied by inactivation of these enzymes. Insulin simultaneously induces activation and dephosphorylation of hepatic acetyl-CoA carboxylase[16] and of pyruvate dehydrogenase in fat cells[25]. It is attractive to speculate that insulin via one common mechanism triggers the dephosphorylation of all the regulatory enzymes of lipogenesis. Unfortunately, experimental evidence for this hypothesis is largely lacking at present.

Interrelationship between short-term hormonal control of lipogenesis and of the other major metabolic processes in the liver

It is now recognized that glycogen metabolism, gluconeogenesis, glycolysis, fatty acid oxidation (ketogenesis), fatty acid synthesis, fatty acid esterification and cholesterogenesis are all coordinately regulated in the liver by short-term regulatory mechanisms. This seems necessary in view of the possible operation of futile cycles between glucose and glycogen and between acetyl-CoA and fatty acyl-CoA. With regard to insulin-mediated control of liver metabolism, a coherent picture is emerging.

In isolated hepatocytes, insulin stimulates glycolysis (Table II), fatty acid synthesis[5,10], glycogen synthesis (Table II), fatty acid esterification[14] and cholesterol synthesis[26]. On the other hand, fatty acid oxidation[14] and gluconeogenesis (Table II) are depressed by insulin. All these metabolic pathways are affected by glucagon in a manner opposite to that of insulin (for review see Ref. 21).

In summary, the regulatory enzymatic sites in the conversion of glucose into fat are rapidly and coordinately activated by insulin, possibly by modulation of their covalent phosphorylation state. Furthermore, insulin induces a rapid decrease in the rates of pathways opposing lipogenesis, i.e. fatty acid oxidation and gluconeogenesis.

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A role for oligosaccharides in glycoprotein biosynthesis

Ron Gibson, Stuart Kornfeld and Sondra Schlesinger

Oligosaccharide chains can influence the ability of a protein to fold properly. The large size of the precursor of asparagine-linked oligosaccharides may be essential if certain proteins are to achieve the correct tertiary structure.

The formation of a functional protein often includes many steps in addition to the synthesis of peptide bonds; one of the most elaborate and perhaps most extensively studied steps is glycosylation. Many membrane and secreted proteins contain oligosaccharide chains covalently attached either to asparagine (N-linked) or to serine and threonine (O-linked). N-linked oligosaccharides are divided into two

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