

Regulation of Acetyl CoA Carboxylase mRNA in Rat Liver by High Carbohydrate Diet and Insulin

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Acetyl CoA carboxylase contents in liver cytosol of rats refed a high carbohydrate diet or injected with insulin were measured by an immunoassay method in order to evaluate the effects of dietary carbohydrate and insulin treatment on the control in the amount of acetyl CoA carboxylase. Acetyl CoA carboxylase was purified 1,552 folds with a specific activity of 3.88 units/mg protein from livers of rats refed a high carbohydrate diet for 3 days following a 3-day fasting and the antibody was generated against the purified acetyl CoA carboxylase in a rabbit. Treatment of insulin (1.5 units/100g BW) and a high carbohydrate diet increased the amount of acetyl CoA carboxylase in liver cytosol by 3 times and 10 times, respectively, when compared to the enzyme content found in the control. The synthetic ratio of acetyl CoA carboxylase to total cytosolic proteins was 4 times higher in the insulin-treated group and 10 times higher in the high carbohydrate diet-treated group than the control group. The polysomal RNA contents in liver cytosols were 279% of the control in the insulin-treated group and 365% of the control in the high carbohydrate diet group. Also, the nascent chain of acetyl CoA carboxylase in polysome were 158% of the control in the insulin-treated group and 311% of the control in the high carbohydrate treated group. From these results, it is assumed that the increase of acetyl CoA carboxylase content in the rat liver cells by insulin treatment, or high carbohydrate diet refeding has resulted from the increased polysomal acetyl CoA carboxylase mRNA, which is directly related to the biosynthesis of this enzyme.

Key Words: Acetyl CoA Carboxylase mRNA, insulin, high carbohydrate diet, immunochemical assay

Acetyl CoA carboxylase (E.C.6.4.1.2) is an enzyme involved in the rate limiting step of fatty acid synthesis in cells (Lane et al, 1974; Volpe and Vagelos, 1976). The long-term regulation of acetyl CoA carboxylase activity in cells is governed by the synthesis and degradation of the enzyme (Majerus and Kilburn, 1969; Nakanishi and Numa, 1970).

The activity of acetyl CoA carboxylase decreases rapidly in tissues of animals undergoing a long-term fasting or in diabetic state, but the enzyme activity increases rapidly when animals are refed a non-fat high carbohydrate diet or when treated with insulin (Nakanishi and Numa, 1970; Kornacker and Lowen-

stein, 1965). Also, when hepatocytes are cultured in a media containing high concentration of glucose without insulin, the acetyl CoA carboxylase activity in the cells is increased and this is due to the increased biosynthesis of the enzyme regardless of its rate of degradation (Katz and Ick, 1981; Giffhorn and Katz, 1984).

Refeeding of rats fasted for long period of time, in general, increases the activities of lipogenic enzymes, and such increase may have resulted from the increased amount of specific mRNA doing for the lipogenic enzyme in cells (Johnson and Sasson, 1966; Gozukara et al, 1972; Wa anabe and Taketa, 1973; Garcia and Holten, 1975; Peavy and Hanssen, 1976; Sun and Holten, 1978). In the liver cell membrane, not like in those of the muscle or adipose cells, insulin does not affect the glucose transport, and controls the activities of enzymes (Golden et al. 1978). It has been reported that the treatment of insulin to the insulin dependent target cells causes a rapid stimulation of cell membrane, alters action potential of membrane, affects the enzyme activity in a few

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minutes, then increases protein synthesis in ribosomes and finally increases the synthesis of DNA and RNA in a few hours (Goldfine, 1981). Insulin receptors are present in nuclear membrane and in microsome, in addition to plasma membrane (Horvat and Katsoyannis 1975; Goldfine and Smith 1976) and insulin receptors in nuclear membrane are also regulated by the concentration of extracellular insulin as in plasma membrane (Vigneri *et al.* 1978). Biosynthesis of fat from non-fat precursors increases in rats refed a high carbohydrate diet, and this is related to the increased susceptibility to insulin of cells (Tepperman and Tepperman, 1964; Leveill, 1972; O'Dea, 1978; Spence *et al.* 1979). Recently, Whang *et al.* (1982) reported that in rats fasted for a long period of time, insulin receptors are accumulated on the plasma membrane of the liver cells, and that when they were refed a high carbohydrate diet, both blood glucose level and insulin secretion were increased. Then in turn insulin binds to insulin receptors, and these receptor-complexes are internalized into cells, and stimulate the synthesis of lipogenic enzymes.

It has been also reported that insulin receptors on nuclear membrane as well as on plasma membrane of liver cells were increased in number in rats fasted for a long period of time.

And when the rats were refed a high carbohydrate diet, the blood glucose and insulin levels are increased (Yoon *et al.* 1983). Similar results on thereby the efflux of mRNA for lipogenic enzymes is increased (Yoon *et al.* 1983). A similar results on glucose-6-phosphate dehydrogenase, and fatty acid synthetase were reported by Kim *et al.* (1985) and Suh *et al.* (1986).

In the present study, acetyl CoA carboxylase, a lipogenic enzyme involved in the rate limiting step of fatty acid synthesis is quantitated by an immunochemical assay method to illustrate the effect of insulin and high carbohydrate diet on the regulation of the biosynthesis of this enzyme.

MATERIALS AND METHODS

Animals and Treatment

Nine Sprague Dawley male rats (150g BW) were divided into three groups: control, insulin-treated and high carbohydrate-diet group.

Rats in the control group received sucrose (2.0g/kg BW) by intragastric intubation and rats in the insulin-treated group were injected intraperitoneally with insulin (1.5 units/100g BW) in addition to the sucrose administration as in the control group. Rats in high

carbohydrate-diet group were refed a high carbohydrate diet (Kim and Lambooy, 1967) for 3 days. All rats were fasted for 3 days before each treatment.

For the purification of acetyl CoA carboxylase from 50 adult rat livers, the enzyme was induced in rats by feeding a high carbohydrate diet for 3 days after fasting for 3 days.

Enzyme assay

The activity of acetyl CoA carboxylase was measured by the method of Giffhorn and Katz (1984). Fresh livers were homogenized with 10 volume (w/v) of 5mM Tris-HCl buffer (pH 7.4) containing 130mM NaCl and 30mM KCl and centrifuged at 12,000xg for 10 min, then the supernatant was used as the enzyme solution.

The solution was adjusted to 5mM MgCl₂, 5mM citrate, 1.25mM 2-mercaptoethanol (2-ME) and 0.1% fat-free albumin (w/v), and incubated for 30min at 37°C.

The concentrations of ATP, acetyl CoA and KH¹⁴CO₃ in above enzymes solution were adjusted to 2.8, 0.15 and 15.6mM, respectively, then incubated for 10min at 37°C. The enzyme reaction was stopped by addition of 0.02ml of 6N HCl to the reaction mixture followed by the centrifugation at 10,000 rpm for 10min in Beckman Microfuge.

The supernatant was transferred into scintillation counting vial and dried in an oven at 80°C. The radioactivity in the residues dissolved in the counting vial was counted by Packard Tri-carb 300 and the specific activity of enzyme was defined as cpm per mg protein. The amount of protein was determined by the method of Lowry *et al.* (1951).

Purification of acetyl CoA carboxylase from rat liver cytosol

Precipitation with polyethylene glycol and ammonium sulfate: Acetyl CoA carboxylase in rat liver was isolated with the modified methods of Nakanish and Numa (1970) and Song and Kim (1981). Fresh liver was minced and homogenized with 2 volumes of 0.1M potassium phosphate buffer (pH 7.5) containing 2mM EDTA, 10mM 2-mercaptoethanol, 0.2mM phenylmethylsulfonyl fluoride (PMSF) and 0.25M sucrose using a Polytron homogenizer.

The homogenate was centrifuged at 20,000xg for 2 hours, and polyethylene glycol (6,000) was added to the supernatant in a final concentration of 3%, followed by standing for 20min at 4°C. The precipitate was removed after the centrifugation at 20,000xg for 20min. To the supernatant, crytallized potassium citrate was added to a final concentration of 10mM and

stirred for 2 hours at 4°C. After the centrifugation at 20,000xg for 30min, polyethylene glycol was added to the supernatant to a final concentration of 5%. After centrifugation again, the precipitate was dissolved in a 0.1M potassium phosphate buffer containing 10mM citrate and the denatured protein was removed by the centrifugation at 10,000xg for 10min. The supernatant containing the enzyme was precipitated with a 40% saturation of ammonium sulfate and the precipitate was redissolved in a 0.1M phosphate buffer containing 10mM citrate, and denatured protein was removed by centrifugation as described above.

Sephacrose 2B gel filtration: Onto a Sepharose 2B column (2.5x90cm) equilibrated with 0.1M phosphate buffer (pH7.5), enzyme solution prepared by the previous step was applied and eluted with the same buffer. Fractions containing acetyl CoA carboxylase activity were pooled and precipitated again with ammonium sulfate (40% saturation). After the centrifugation at 20,000xg for 10min the precipitate was dissolved in 20mM potassium phosphate (pH7.4) containing 10mM citrate 5mM 2-mercaptoethanol and 1mM EDTA and dialyzed for 12 hours at 4°C against the same buffer. The dialyzed sample was stored at -70°C until use.

SDS polyacrylamide gel electrophoresis (PAGE)

SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresis of the purified acetyl CoA carboxylase was carried out by the method of Laemmli (1970). The samples dissolved in 6.25mM Tris-HCl buffer (pH6.8) containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.1% bromophenol blue were boiled for 3 min in water bath, and then cooled. Each 50ug of sample was applied onto each well of the 7% polyacrylamide slab gel containing 0.1% SDS and then electrophoresed at a constant current (10mA for stacking, 15mA for separating). After the electrophoresis, the gel was stained with 0.1% Coomassie brilliant blue (R-250) in 50% methanol and 12.5% trichloroacetic acid for 18 hours, and destained with a solution made of 30% methanol and 10% acetic acid.

Preparation of IgG against acetyl CoA carboxylase

A Rabbit was immunized with acetyl CoA carboxylase by the method of Spence *et al.* (1985). 100ug of pure acetyl CoA carboxylase (0.2ml) was emulsified with equal volume of complete Freund's adjuvant and injected into a rabbit subcutaneously, then the booster injection was carried out after 4 weeks with 100ug of the enzyme emulsified with equal volume of incomplete Freund's adjuvant. After 2 weeks from the booster injection, blood was drained and serum

was collected by centrifugation.

Immunodiffusion test was carried out by the method of Oka *et al.* (1984). Agar (1%) plate with 6 wells around a center well was prepared, and double immunodiffusions between antiserum and acetyl CoA carboxylase prepared from each purification step were tested. The precipitins formed were visualized with 2.5% Coomassie brilliant blue (R-250) dissolved in 45% ethanol and 10% acetic acid for 10 minutes followed by the destaining with a solution made of 45% ethanol and 10% acetic acid.

IgG was purified from antiserum by a protein A Sepharose CL-4B (Sigma) column chromatography according to the method of Kraus and Rosenberg (1982). Onto a protein A-Sepharose column (1x5cm) equilibrated with 0.1M potassium phosphate buffer (pH7.0) the diluted antiserum with 0.1M potassium phosphate buffer (pH7.0) (1:1 dilution) was laid and washed with 60ml of the same buffer followed by the elution of IgG with 0.1M glycine buffer (pH 3.0). IgG fractions were collected and immediately neutralized with 20mM sodium phosphate buffer (pH 7.6), containing 150mM NaCl and dialyzed for 18 hours at 4°C against the same solution. The dialyzed IgG solution was concentrated to 2ml by dry dialysis with Dextran T250 (Pharmacia Fine chemicals) and stored at -15°C in 50% glycerol (v/v).

Immunochemical assay of acetyl CoA carboxylase

The titer of anti-acetyl CoA carboxylase antibody (IgG) was determined by the method of Nakanish and Numa (1970). 200μl (12mg/ml) of enzyme solution prepared from the 5% polyethylene glycol precipitation as described earlier was added to each tube containing 0, 10, 20, 40, and 80μl of IgG (6.5mg/ml), diluted to 300μl with homogenate buffer solution and then left standing at 4°C for 1 hour.

Then 50μl of 10% heat-killed formaldehyde-fixed *Staphylococcus aureus*, Cowan I (SaC), was added to each mixture followed by another standing for 30 min at 4°C.

The content was centrifuged for 5 min with Microfuge and the acetyl CoA carboxylase activity in the supernatant was measured by the method of Giffhorn and Katz (1984).

Quantitative determination of acetyl CoA carboxylase

Acetyl CoA carboxylase content in rat liver cytosol was measured by a modified method of Spence *et al.* (1985). Rats in each group (control, insulin-treated, high carbohydrate-diet) were injected with [³H] leucine (100μCi, specific activity 130-190 Ci/mmol) in-

traperitoneally after each treatment.

At 30 min after the injection of [^3H] leucine, rats were sacrificed and livers were removed immediately, chilled and homogenized with 5.5 volume (w/v) of 0.25M sucrose containing 50mM Tris-HCl buffer (pH 7.5), 25mM NaCl, 5mM MgCl_2 , 20mg% heparin and 0.1mg cycloheximide.

The homogenate was centrifuged at 10,000g for 10 min at 4°C in a Beckman Ti 75 rotor. The supernatant represents cytosol. To each 2ml of cytosol, 40 μl of serum (normal serum or antiserum for acetyl CoA carboxylase) was added and incubated for 1 hours at 37°C followed by the addition of 120 μl of 10% SaC and further incubation for 30 min at 37°C. The antigen-antibody complex was pelleted by the centrifugation with Microfuge for 5 min and the precipitate was washed 4 times with 500 μl of 10mM Tris-HCl buffer (pH 7.5), containing 140mM NaCl, 2.5mM EDTA, 5mM leucine, 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.5% SDS and 1mM phenylmethylsulfonylfluoride. To the washed precipitates, 150 μl of 50mM Tris-HCl buffer (pH 6.8) containing 10% SDS, 1% 2-mercaptoethanol, and 10% glycerol were added and heated for 5 min in boiling water bath.

The content was centrifuged for 5 min with Microfuge and the each 120 μl of the supernatant was transferred into the scintillation counting vials containing 15ml of scintillation cocktail solution and the radioactivities incorporated into acetyl CoA carboxylase were counted.

Isolation and quantitation of polysomal RNA for acetyl CoA carboxylase

Liver homogenate (15%) from each group of rats was centrifuged for 10 min at 14,500 rpm with Sorvall SS-34 rotor and the supernatant was mixed with 0.1 volume of 10% (w/v) sodium deoxycholate and 10% (v/v) Triton X-100, then the mixture was subjected to the discontinuous sucrose density gradient cen-

trifugation by the method of Kraus and Rosenberg (1982).

Beginning from the bottom of Beckman SW 41 rotor centrifuge tube, 2ml of 2.5M sucrose solution containing 25mM Tris-HCl buffer (pH 7.5), 150mM NaCl, 5mM MgCl_2 , 20mg% heparin and 0.1mg% cycloheximide, then 4ml of 1.0M sucrose and finally 6ml of liver supernatant was overlaid on the top.

The tubes were centrifuged for 100min at 40,000 rpm, and polysomal bands formed between two sucrose layers were carefully removed and mixed with an equal volume of polysomal buffer containing 25mM Tris-HCl (pH 7.5), 150mM MgCl_2 , 20mg% heparin and 0.1mg% cycloheximide and 0.1% Nonidet P-40 then dialyzed for 18 hours at 4°C against the same buffer and stored at -80°C. For the measurement of polysomal RNA specific for acetyl CoA carboxylase, the frozen polysomes were thawed at room temperature and centrifuged for 10 min at 10,000g.

The supernatants were removed and 2ml portions were delivered into each test tube in triplicate and 40 μl of normal serum or antiserum for acetyl CoA carboxylase were added to each tube.

The reaction mixtures were incubated for 60 min at 37°C and 120 μl of 10% SaC were added and incubated for another 30min at 37°C. After incubation, the mixtures were centrifuged for 5 min with Microfuge and the precipitates were washed 4 times with each 1ml of polysomal buffer and dissolved in 1ml of 25mM Tris-HCl buffer (pH 7.5) containing 20mM EDTA and 20mg% heparin followed by the standing at room temperature for 60 min. The dissolved samples were centrifuged with Microfuge for 10 min and absorbance of the supernatants were read at 260nm.

Polysomal protein precipitated with the antiserum for acetyl CoA carboxylase was measured by counting the radioactivity of [^3H] leucine incorporated into the polysomal proteins in 400 μl of supernatant released from the antigen-antibody complex by the incubation with 1ml of 0.1M glycine buffer (pH 3.0) for 30 min.

Table 1. Purification profiles of acetyl CoA carboxylase from rat liver

Purification step	Volume (ml)	Protein (mg/ml)	Activity (total)	Specific activity* (unit/mg)	Folds
1) Homogenates (20,000 \times g supernatant)	800	36.9	72.3	0.0025	1
2) 3% Polyethylene glycol-extract	800	28.4	72.0	0.0032	1.28
3) 5% Polyethylene glycol extract	370	9.7	26.9	0.0075	3.00
4) 40% Ammonium sulfate fraction	330	3.0	23.3	0.0236	9.44
5) Sepharose 2B column chromatography	72	0.127	29.5	3.880	1552

* μ moles of malonyl CoA produced/10 min/mg protein.

RESULTS

Purification of acetyl CoA carboxylase

Acetyl CoA carboxylase (9.14mg) was purified from 250g of liver of rats fed with a fat free-high carbohydrate diet for 3 days after the 3 days of fasting.

The enzyme activities of fraction number 36-50 eluates from Sepharose 2B column were coincided with protein peak, and the purified enzyme obtained from these fractions had a specific activity of 3.88 units/mg protein with overall 1552 folds purification (Table 1).

Sepharose 2B column chromatography (final purification step) of the enzyme solution was an extensive step for the purification (Fig. 1), and SDS-PAGE of the purified acetyl CoA carboxylase eluate of fraction number 36-50 showed 3 protein bands corresponding to the molecular weight of 220,000, 120,000 and 50,000 daltons (Fig. 2).

Preparation of antiserum for acetyl CoA carboxylase

Ouchterlony double immunodiffusion test of antiserum for acetyl CoA carboxylase and 20,000xg su-

pernatant of liver homogenate or 3% polyethylene glycol precipitate revealed a sharply defined, continuous hexagonal precipitin, indicating the formation of the identical antiserum (Fig. 3).

IgG isolated from antiserum for acetyl CoA carboxylase by protein A-Sepharose column chromatography showed a single protein peak (Fig. 4), and the IgG content in the pooled elution from fraction No 3-7 was 6.5mg/ml.

Effects of insulin and high carbohydrate diet on the acetyl CoA carboxylase content in rat liver cytosol

Immunochemical titration of acetyl CoA carboxylase in 5% polyethylene glycol precipitate with various amount of IgG isolated from antiserum for acetyl CoA carboxylase showed gradual inhibition of the enzyme activity in proportion to the IgG concentrations (Fig. 5). Acetyl CoA carboxylase contents in rat liver cytosol of the control group, insulin-treated group and high carbohydrate diet-treated group were $1,036 \pm 263$, $3,308 \pm 634$ and $10,084 \pm 1031$ dpm, respectively (Table 2).

The biosynthetic ratio of the enzyme to the total cytosolic proteins in the control group, insulin-treated group and high carbohydrate-treated group were

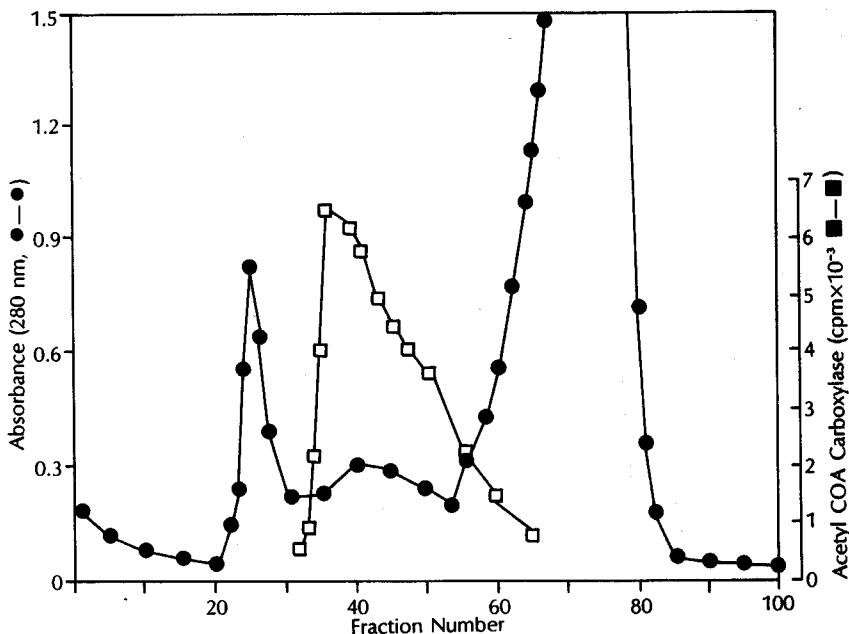


Fig. 1. Sepharose 2B column chromatography. Bed volume of Sepharose 2B column was 400 ml. Enzyme solution was applied to the column which had been equilibrated with 0.1M potassium phosphate buffer, pH7.5 containing 2mM EDTA, 10mM β -mercaptoethanol, 10mM citrate, and the column was eluted with the same phosphate buffer.

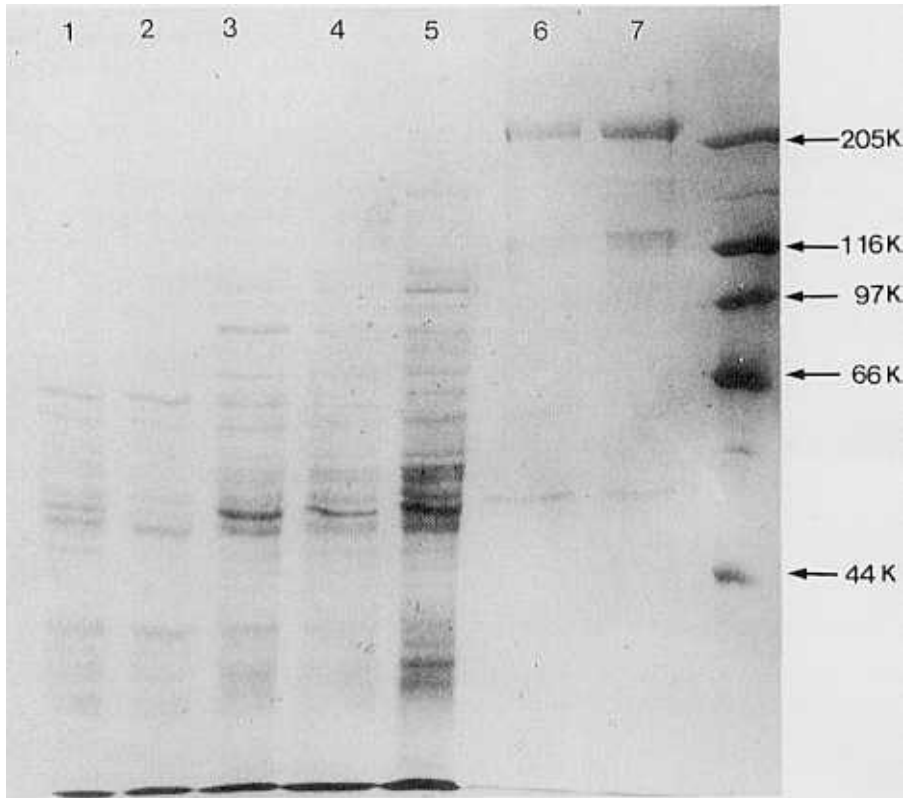


Fig. 2. SDS-polyacrylamide gel electrophoretic pattern of acetyl CoA carboxylase.

Lane 1. High speed centrifugation supernatant.

2. 3% polyethylene glycol extracts.

3. 5% polyethylene glycol fraction.

4. 0.1M phosphate buffer (pH 7.5) extracts.

5. 40% ammonium sulfate fraction.

6. 7. After Sepharose 2B gel filtration.

8. Standard proteins, myosin (205 kd), β -galactosidase (116 kd), phosphorylase b (97 kd), bovine serum albumin (66 kd) and ovalbumin (44 kd).

1.57×10^{-3} , 6.85×10^{-3} , and 15.6×10^{-3} , respectively.

These data indicate that insulin and high carbohydrate diet stimulate the biosynthesis of acetyl CoA carboxylase.

Effects of insulin and high carbohydrate diet on the amount of polysomal RNA specific for acetyl CoA carboxylase in rat liver cytosol

The content of polysomal RNA specific for acetyl CoA carboxylase precipitated with antiserum for acetyl CoA carboxylase in the control, insulin-treated and high carbohydrate diet-treated group were 1.33 ± 0.40 , 3.75 ± 0.56 and 4.89 ± 0.52 units, respective-

ly, per 1,000 units of the total polysomal RNA (Table 3).

This result indicates that high carbohydrate diet and insulin treatment increase the amount of polysomal mRNA specific for acetyl CoA carboxylase, thereby induce the enzyme biosynthesis and increase the enzyme activity in the rat liver cytosol.

Effect of insulin and high carbohydrate diet on the amount of acetyl CoA carboxylase in rat liver cytosol

Radioactivities of [3 H] leucine incorporated into rat liver polysomal protein precipitated with antiserum for acetyl CoA carboxylase, in the control, insulin-

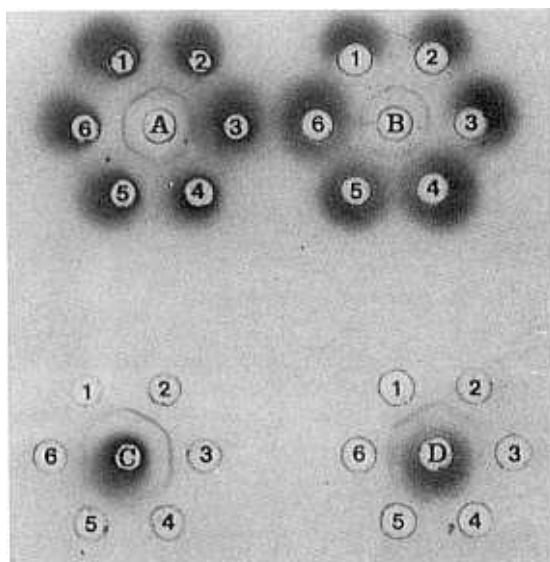


Fig. 3. Double immunodiffusion pattern of rabbit antiserum and acetyl CoA carboxylase from rat liver. The method was described in the text.

A,B: Anti-serum 15 μ l.

1,2,3,4,5,6 around A; 20,000 \times g liver supernatant solution (500 μ g protein/15 μ l).

1,2,3,4,5,6 around B; 3% polyethylene glycol extract (400 μ g protein/15 μ l).

C; 20,000 \times g liver supernatant solution.

D; 3% polyethylene glycol extract.

1,2,3,4,5,6 around C and D; anti-serum 15 μ l.

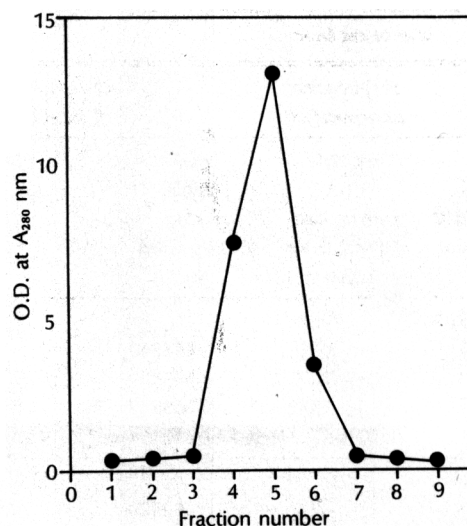


Fig. 4. Protein A Sepharose CL-4B chromatography of rabbit anti-serum.

Anti-serum (3-6 ml) was mixed 1:1 (vol/vol) with 0.1M potassium phosphate buffer (pH 7.0) and applied to a 1 \times 5 cm column of protein A-Sepharose CL-4B (Sigma). The column was washed with 60ml of 0.1 M potassium phosphate buffer (pH 7.0) and the IgG fraction was eluted with 0.1M glycine buffer (pH 3.0).

treated and high carbohydrate-treated group were $1,827 \pm 172$, $2,805 \pm 88$ and $5,694 \pm 751$ dpm, respectively (Table 4).

The result indicates that the acetyl CoA carboxylase nascent chains in the liver polysomes were increased 153% by insulin treatment and 311% by high carbohydrate diet feeding.

DISCUSSION

The present study was undertaken in order to elucidate the mechanism of the regulation of acetyl CoA carboxylase, a lipogenic enzyme, by high carbohydrate diet and insulin.

The degree of acetyl CoA carboxylase biosynthesis was estimated by the quantitation of nascent chains synthesized from polysomal mRNA specific for the enzyme by the immunoprecipitation with antiserum for acetyl CoA carboxylase.

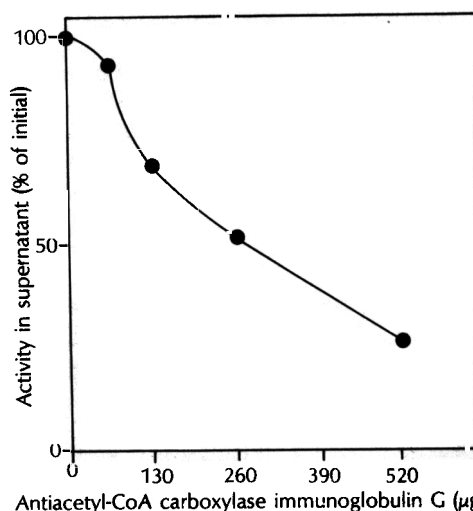


Fig. 5. Immunochemical titration of liver acetyl CoA carboxylase from refed rat. Increasing amounts of anti-acetyl CoA carboxylase immunoglobulin G were added to 0.2ml of 40% ammonium sulfate fraction (see materials and methods). Following the completion of precipitation, the supernatant fluids were assayed for acetyl CoA carboxylase activity at 37°C.

Table 2. Relative rate of synthesis for acetyl CoA carboxylase in rat liver

Animal group	[³ H]-Leucine incorporation	Relative rate of synthesis	
	Acetyl CoA carboxylase (DPM/g liver) [A]	Total soluble protein (DPM/g liver) [B]	A/B×10 ³
Control			
1	728	618660	
2	1008	694820	
3	1372	669240	
	1036±263*	660660±10154	1.57
Insulin			
1	4220	482020	
2	2772	492780	
3	2954	472640	
	3308±634	482480±8228	6.85
Induction			
1	9226	640640	
2	11536	658980	
3	9492	636300	
	10084±1031	645306±9829	15.6

*Mean±S.D.

The purity of the enzyme used in the present study was about 95% as evidenced from the SDS-PAGE analysis, which showed the major protein bands corresponding to molecular weights of 220,000, 120,000 and 50,000 daltons.

Nakanishi and Numa (1970) had reported acetyl CoA carboxylase purified from 200 rat livers (1660g), which had a specific activity of 7.55 units/mg protein (total yield 10.2mg). It has also been reported that acetyl CoA carboxylase purified from chicken livers had a specific activity of 3-5 units/mg protein (Numa *et al.* 1966; Goto *et al.* 1967; Numa 1969).

Acetyl CoA carboxylase purified from 250g of rat livers in the present study has a specific activity of 3.88 units/mg protein (total yield 9.14 mg), which is about 50% of that reported by Nakanishi and Numa (1970) but with much higher total enzyme yield.

The discrepancy between the present results and previous reports might be due to the difference in the enzyme purification procedures and the enzyme assay system. Nakanishi and Numa (1970) purified the enzyme through a calcium phosphate gel fractionation, ammonium sulfate precipitation, DEAE-cellulose

Table 3. Level of specific polysomal RNA for acetyl CoA carboxylase in rat livers by dietary induction and insulin treatment rat liver

Animal group	Specific polysomal RNA (units/A ₂₆₀ 1000 units)	Percent increase
Control		
1	0.833	
2	1.833	
3	1.333	
	1.333±0.40*	100
Insulin		
1	3.514	
2	3.223	
3	4.531	
	3.756±0.56	279
Induction		
1	4.524	
2	5.632	
3	4.514	
	4.890±0.52	365

*Mean±S.D.

Table 4. Level of specific polysomal protein precipitated by antiserum for acetyl CoA carboxylase

Animal group	Specific polysomal protein (DPM/g liver)	Percent increase
Control		
1	2070	
2	1683	
3	1728	
	1827±172*	100
Insulin		
1	2916	
2	2799	
3	2700	
	2805±88.3	153
Induction		
1	5598	
2	4825	
3	6659	
	5694±751	311

*Mean±S.D.

column chromatography and Sepharose 2B gel filtration chromatography and acetyl CoA carboxylase activity measured through a coupling system of pyruvate kinase-lactate dehydrogenase, while in the present study the enzyme was purified through a polyethylene glycol extract procedure and enzyme activities were measured with radioactive products, directly.

Nakanishi and Numa (1970) have reported to molecular weight (MW) of acetyl carboxylase purified from rat liver as 230,000 (major) and 120,000 (minor) daltons. However, Sung and Kim (1981) have reported MW as 260,000 daltons by different enzyme purification procedures. They carried out the purification process as rapidly as possible in the presence of protease inhibitor, phenylmethylsulfonylfluoride. The MW of acetyl CoA carboxylase is approximated as 230,000-260,000 daltons which may be hydrolyzed into small molecules by hydrolytic enzymes in the cell.

The similar results have been reported by several other investigators (Inoue and Lowenstein, 1972; Tanabe *et al.* 1975; MacKall and Lane, 1977).

It has been reported that partial hydrolysis of acetyl CoA carboxylase by proteolytic enzymes resulted in marked elevation of enzyme activity (Iritani *et al.* 1969). And a limited trypsin digestion of acetyl CoA carboxylase increased the rates of incorporation of acetyl CoA into fatty acid (Swanson *et al.* 1967).

The MW of 220,000 and 120,000 of acetyl CoA carboxylase purified in the present study might have resulted from the partial hydrolysis of the enzyme protein during the purification procedures, and marked elevation of the enzyme activity in final purification is probably due to the small fragment of enzyme.

Antiserum for acetyl CoA carboxylase produced in this study cross reacted with the enzyme in liver homogenate supernatant and 3% polyethylene glycol precipitate and formed a single continuous hexagonal precipitin, indicating that the prepared antiserum is specific for the enzyme. Titration of acetyl CoA carboxylase in liver cytosol with IgG prepared from antiserum for acetyl CoA carboxylase showed the gradual inhibition of the enzyme activity with the increase of amount of IgG, indicating that the IgG prepared in the present study was specific for acetyl CoA carboxylase.

Acetyl CoA carboxylase content in the liver cytosol increased 3 folds by insulin treatment and 10 folds by the high carbohydrate diet in the present study, and the biosynthetic ratio of acetyl CoA carboxylase relative to the total liver cytosolic protein increased 4 folds by insulin treatment and 10 folds by refeeding a high carbohydrate diet. The result indicates that insulin and high carbohydrate diet stimulate the bi-

osynthesis of acetyl CoA carboxylase in the liver cytosol and supports the report of Giffhorn and Katz (1984). They reported that the increase of acetyl CoA carboxylase activity by the addition of insulin and glucose to the primary hepatocyte culture resulted from the increased biosynthesis of the enzyme.

It has been reported that insulin administration to the experimental diabetic rats caused a marked elevation of acetyl CoA carboxylase activity, probably by the elevated synthesis of the enzyme (Spence *et al.* 1985). The level of insulin administered in this study was much higher than that applicable to human subject, because 15 units/kg/day of insulin was required for the response to streptozotocin-induced diabetic rats (Davidson and Kaplan 1977).

Instead, 0.4 units/kg/day was sufficient for the human (Foster 1983). Recently, Giffhorn and Katz (1984) and Giffhorn-Katz and Katz (1986) reported that 100nM of insulin and 20mM glucose could induce acetyl CoA carboxylase and fatty acid synthetase in hepatocyte culture system.

The variation in the amount of insulin to get the response may arise from the difference in the susceptibility and number of insulin receptor.

The elevation of the amount of polysomal mRNA specific for acetyl CoA carboxylase and the acetyl CoA carboxylase nascent chains in polysomes by insulin and high carbohydrate diet in the present study is well agreed in part with the results of Nakanishi *et al.* (1976) and Finkelstein *et al.* (1979). It has been reported that insulin and glucose induce acetyl CoA carboxylase including other lipogenic enzymes through long term regulation in cultured hepatocytes (Spence and Pitot 1982). Insulin administration to rats fasted for 2 days increased polysomal mRNA for fatty acid synthetase and glucose-6-phosphate dehydrogenase by means of increased efflux of mRNA from nucleus to cytosol (Lee *et al.* 1987). It is believed that increased polysomal mRNA by insulin might be related to insulin receptors in nuclear membrane. When nuclei of hepatocytes were treated with 1% Triton X-100, the insulin effects disappear. This observation strongly supports the idea that insulin binds to insulin receptors on nuclear membrane and stimulates the transport of mRNA to cytosol than mRNA processing in nucleus (Schum and Webb, 1981). The increased efflux for fatty acid synthetase by insulin treatment has been demonstrated in isolated nucleus of liver of rat fasted for 3 days (Yoon *et al.* 1983).

The elevating effects of insulin on various cytosolic mRNA contents were demonstrated in various tissues or liver cells (Steiner and King, 1966; Pilkis and Salaman, 1972; Peavy *et al.* 1978; Roy *et al.* 1980;

Bolander *et al.* 1981; Korc *et al.* 1981; Peavy *et al.* 1978; Roy *et al.* 1980; Bolander *et al.* 1981; Korc *et al.* 1981; Pry and Porter, 1981). From this and previous studies, it is concluded that the increase of acetyl CoA carboxylase activity in liver cytosol of rats by insulin or high carbohydrate diet is due to the increased biosynthesis of polysomal mRNA for the enzyme.

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