CHAPTER-I
Alcoholism and its Impact on Carbohydrate Metabolism
INTRODUCTION

There has been a significant rise of alcohol consumption due to increased urbanization and easy availability of alcohol in recent times. The incidence of diabetes mellitus has also been observed to be increased at par with changes in life style in conjunction with dietary pattern. In man, excessive alcohol consumption is associated with the appearance of degenerative fibrotic hepatic disease cirrhosis, which is one of the leading causes of alcohol-induced mortality (Smucker, 1975; Larkin and Watson-Williams, 1984). Studies on the link between alcohol consumption and glycoregulation revealed that alcohol consumption might be a target for primary and secondary prevention of impaired glycoregulation and diabetes mellitus (Lombrail et al., 1992; Xu et al., 1998). Large intake of alcohol led to the development of frank clinical diabetes mellitus with glucose intolerance. Hazard of heavy alcohol intake induces hyperglycemia, glucose intolerance, inhibition of insulin secretion, increased insulin resistance and hypertriglyceridemia (Yki et al., 1985; Avogaro et al., 1987; Lomeo et al., 1988). In the postprandial state alcohol induces hyperglycemia by inducing glycogenolysis and accelerate the peripheral insulin resistance (Bell, 1996; Ahmed, 1995; Walsh and O’ Sullivan, 1974). Heavy alcohol intake is associated with glucose intolerance caused by an inhibition of insulin secretion and increased insulin resistance at both the receptor and post-receptor levels (Holley et al., 1981; Tiengo et al., 1981; Ben et al., 1991; Koivisto et al, 1993). Though alcohol abuse is thought to be a risk factor for the cause of liver damage, hyperlipidemia and insulin resistance nevertheless, the mechanisms by which alcoholic beverages could mediate insulin resistance in target organs are not very well known due to lack of suitable experimental models.

In view of the conflicting results obtained in various studies, a prospective longitudinal study has been made in albino rats, the most commonly used animals in toxicity research to ascertain complex metabolic effects of ethanol on insulin resistance.
MATERIALS AND METHODS

Chemicals

Folin ciocalataeu's phenol reagent, fructose, sodium pyruvate, oxaloacetate, tris HCl, potassium chloride, magnesium chloride, adenosine triphosphate and adenosine diphosphate were procured from Sisco Research Laboratory (SRL), Bombay. Bovine serum albumin, adenosine monophosphate, NADH, NADPH, glycogen, PEP, LDH, MDH, glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, sodium fluoride, EDTA, aldolase and triosephosphate isomerase were purchased from Sigma chemical company, St. Louis, USA. Sucrose and glucose were purchased from Himedia, Bombay. Ethanol, methanol, chloroform, sodium acetate, sulphuric acid, hydrochloric acid, acetic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, citric acid and sodium citrate were purchased from E. Merck, Germany. The Radioimmunoassay kits for the measurements of insulin, C-peptide, GLP-1, leptin and adiponectin were purchased either from Bhabha Atomic Research Centre (BARC), Bombay, India or Linco Research Inc, USA. All other kits used for estimation of other biochemical parameters in serum were procured from either Roche or Accurex chemical company. All other chemicals and solvents used were of highest purity grade.

Experimental Protocols

Alcoholism in Rats

Male Charles Foster strain of albino rats aged 50 ± 2 days and body weight 120 ± 20 g were used in the present study. The rats were randomly divided into groups. Group I termed as control rats, which were given sucrose (10 %) in addition to normal pellet diet; this was done primarily to maintain isocaloric conditions. Group II termed as alcoholic rats received ethanol at dose of 3.76 g/kg body weight per day for 45 consecutive days. Oral glucose tolerance test of each rat was made on day 0, 14, 21, 28, 35 and 42 to check the impairedness in the glucose tolerance.

The blood of each animal (overnight fasted) was collected on day 46th and the animals were finally sacrificed by decapitation on the same day and the liver, kidney and muscle of each animal was quickly excised and stored at -80°C until use for the estimations of various enzyme activities. Each biochemical parameter was expressed as
mean ± standard deviation. Groups were compared by analysis of variance with the Student's 't' test.

Biochemical Parameters

Aspartate Amino Transferase (DL-Aspartate : 2-oxoglutarate Amino Transferase; EC 2.6.1.1)

Aspartate aminotransferase was estimated using kit provided by Roche Chemical Company. α-ketoglutarate reacts with L-Aspartate in the presence of aspartate aminotransferase (AST) to form oxaloacetate and L-glutamate. The increase in oxaloacetate is determined in an indicator reaction catalyzed by malate dehydrogenase. The conversion of NADH to NAD⁺ is proportional to the AST activity in the specimen and determined kinetically.

Alanine Amino Transferase (DL-Alanine : 2-oxoglutarate Amino Transferase EC 2.6.1.2)

Alanine aminotransferase was estimated using kit provided by Roche Chemical Company. α-ketoglutarate reacts with L-Alanine in the presence of alanine aminotransferase (ALT) to form pyruvate and L-glutamate. The increase in pyruvate is determined in an indicator reaction catalyzed by lactate dehydrogenase. The conversion of NADH to NAD⁺ is proportional to the ALT activity in the specimen and determined kinetically.

γ-Glutamyl Transpeptidase (γ-Glutamyl Transferase; EC 2.3.3.2)

γ-Glutamyl transpeptidase was estimated using kit provided by Roche Chemical Company. γ-Glutamyl transpeptidase (γ-GT) transfer the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate liberated is proportional to the γ-GT activity in the specimen and was measured kinetically.

Alkaline Phosphatase (Orthophosphoric Monoester Phosphohydrolase; EC 3.1.3.1)

Alkaline phosphatase was estimated using kit provided by Roche Chemical Company. Alkaline phosphatase cleaves p-nitrophenyl phosphate (p-NPP) in the presence of magnesium ions to form p-nitrophenol and phosphate. The intensity of
yellow colour formed is directly proportional to the alkaline phosphatase activity in the specimen and measured kinetically.

**Acid Phosphatase (Orthophosphoric Monoester Phosphohydrolase; EC 3.1.3.2)**

The enzyme activity was determined by the method of Wright *et al.* (1972). To 3.0 ml of assay system, consisted of 43 mM acetate buffer (pH 4.5), 2.33 mM p-nitrophenyl phosphate and suitable diluted serum enzyme. After 15 min of incubation at 37°C, the reaction was terminated with 2.0 ml of 1.0 N sodium hydroxide. The intensity of the colour was read at 405 nm.

**Sorbitol Dehydrogenase (Sorbitol: NAD - Oxidoreductase; EC 1.1.1.14)**

Sorbitol dehydrogenase was assayed according to the method of Gerlach and Hiby as reported in Bergmeyer (1974). The reaction mixture contained 500 mM tris-HCl buffer (pH 7.4), 0.2 mM NADH₂, 0.3 ml Fructose (72 % w/v) and 100-200 µg enzyme protein. Optical density change was observed at 340 nm for 3 min at 30 sec intervals.

**Alcohol Dehydrogenase (EC 1.1.1.1)**

Alcohol dehydrogenase was estimated by the method of Agarwal and Goedde (1990). The reaction mixture contained 0.1 M Glycine-NaOH buffer (pH 9.6), 0.1 ml NAD (10 mg/kg), 0.1 ml ethanol (95 %). 0.1 ml of suitably diluted serum were added and rise in optical density at 340 nm was recorded for 3 min at 30 sec intervals.

**Bilirubin**

Serum bilirubin was estimated according to the method of Jendrassik and Grof (1938). The serum sample (0.2 ml) was mixed with 0.2 ml of sulphamonic acid (0.029 mol/l in 0.17 N HCl), 50 µl sodium nitrite (25 mmol/l) and 1.0 ml caffeine reagent (0.26 M caffeine and 0.52 M sodium benzoate in water). The reaction mixture was incubated in a dark place at 25°C for 30 min. After incubation 1.0 ml of Na-K tartarate (0.93 M in 1.9 N NaOH) was added and kept for 15 min at room temperature. The absorbance of the colour developed was measured at 578 nm against a reagent blank and compared with appropriate standard of pure bilirubin.
Cholesterol

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CO) to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with hydroxybenzoic acid (HBA) and 4-aminoantipyrine (4AAA) in the presence of peroxidase (POD) to form a chromophore (quinoneimine dye) which may be quantitated at 500-505 nm. The intensity of red colour formed is directly proportional to the concentration of total cholesterol in the specimen and measured spectrophotometrically (Searcy, 1969; Ellefson and Caraway, 1976).

Triglycerides

Lipoprotein lipase hydrolyses triglycerides to yield glycerol and fatty acids. Glycerol kinase converts glycerol to glycerol-3-phosphate, which is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and 4-chlorophenol to produce red quinonimine dye. The intensity of red colour formed is directly proportional to the concentration of triglycerides in the specimen and is measured photometrically (Wahlefeld et al., 1974).

HDL-Cholesterol

Cholestest N HDL is a liquid reagent that directly measures the HDL-cholesterol concentration in serum by a new method that is based on the selective solubilizing effect of proprietary detergent to the different lipoproteins. In the assay system, only HDL is solubilized by a special detergent; other lipoproteins are not disrupted. After HDL is selectively disrupted, HDL cholesterol is measure enzymatically (Gordon et al., 1977).

LDL-Cholesterol

Cholestest LDL is a liquid reagent that directly measures the concentration of LDL-cholesterol by a new homogeneous method based on an innovative detergent technology. (Nakumara et al., 1997).
Apolipoprotein A-II (APO A-II)

APO A-II kit, utilizing the turbidimetric immunoassay (TIA) technique, enables the quick and accurate measurement of serum (plasma) apolipoprotein A-II level. Antigen-antibody reaction occurs between apolipoprotein A-II in serum and anti-human apolipoprotein A-II antibody, which causes turbidity. Apolipoprotein A-II concentration can be obtained by measurement of turbidity (Sakai et al., 1984; Itakura et al., 1986).

Apolipoprotein C-II (APO C-II)

APO C-II kit, utilizing the turbidimetric immunoassay (TIA) technique, enables the quick and accurate measurement of serum (plasma) apolipoprotein C-II level. Antigen-antibody reaction occurs between apolipoprotein C-II in serum and anti-human apolipoprotein C-II antibody, which causes turbidity. Apolipoprotein C-II concentration can be obtained by measurement of turbidity (Sakai et al., 1984; Itakura et al., 1986).

Apolipoprotein E (APO E)

APO E kit, utilizing the turbidimetric immunoassay (TIA) technique, enables the quick and accurate measurement of serum (plasma) apolipoprotein E level. Antigen-antibody reaction occurs between apolipoprotein E in serum and anti-human apolipoprotein E antibody, which causes turbidity. Apolipoprotein E concentration can be obtained by measurement of turbidity (Sakai et al., 1984; Itakura et al., 1986).

Ethanol

The ethanol was estimated using kit provided by Roche chemical company. To 3.0 ml of reaction mixture A1, 0.1 ml serum sample were added, mixed it well for 3.0 min and read absorbance of the solution A1. Now 0.050 ml solution of A2 were added, mixed well and kept for 10 min. After completion of reaction absorption of the reaction mixture was recorded. Absorbance differences were determined and ethanol concentration in the sample was calculated (Bucher et al., 1951; Beutler and Michal, 1977).
Blood Glucose

Blood glucose was always estimated by glucometer using gluostrips (Boehringer Mannheim).

Serum Insulin

Serum insulin was estimated using the radioimmunoassay kit (RIAK-1) for serum insulin of rat provided by Linco Research Inc, USA, by the following method. EDTA phosphate buffer 0.3 ml (0.01 M EDTA, 0.04 M phosphate, pH 7.4), containing 0.5 % BSA (w/v) was mixed with 0.1 ml of standard insulin or unknown samples and 0.1 ml of antibody (anti-insulin guineapig serum diluted 1:1,000,00 times) and kept overnight at 4°C. 0.1ml of $^{125}$I-insulin (about $1\times10^6$ cpm) was then added to the mixture and was again kept for 20 min at 4°C. Subsequently, 0.1 ml of the second antibody (anti-guineapig-globulin raised in rabbit) was added. Antibody bound reactivity was separated from that of unbound one by precipitation in 12 % polyethylene glycol and counted in Minigamma counter [Wallac, LKB] (Morgan and Lazaro, 1963; Thorell and Lanner, 1973).

C-Peptide

Serum C-peptide was estimated using the radioimmunoassay kit (RIAK-1) for serum C-peptide of rat provided by Linco Research Inc, USA.

Glucagon Like Peptide (GLP-1)

Serum GLP-1 was estimated using the radioimmunoassay kit (RIAK-1) for GLP-1 provided by Linco Research Inc, USA.

Leptin

Serum leptin was estimated using the radioimmunoassay kit (RIAK-1) for leptin provided by Linco Research Inc, USA.

Adiponectin

Serum adiponectin was estimated using the radioimmunoassay kit (RIAK-1) for adiponectin provided by Linco Research Inc, USA.
Preparation of Tissues Homogenate

The overnight-starved animals were sacrificed by cervical dislocation after 45 days of sucrose and ethanol feeding. The liver, kidney and muscle were quickly excised, washed thoroughly with chilled normal saline and kept at −80°C until used. Tissues were taken out and thawed at the time of enzymatic studies. A 10% homogenate of liver, kidney and muscle was prepared in 150 mM KCl with the help of Potter Elvejhem homogenizer fitted with Teflon pestle. The crude homogenates were centrifuged at 3000×rpm for 20 min at 4°C; the clear supernatant was used for determining the activities of glucose-6-phosphatase, glycogen phosphorylase, phosphofructokinase, phosphoenolpyruvate carboxykinase, pyruvate kinase, lactate dehydrogenase, alcohol dehydrogenase and protein tyrosine phosphatase.

Glucose-6-phosphatase (D-Glucose-6-phosphate Phosphorylase; EC 3.1.3.9)

Glucose-6-phosphatase activity was determined according to the method of Hubscher and West (1965). The assay system contained 0.3 M citrate buffer (pH 6.0); 28 mM EDTA, 14 mM NaF, 400 μl water and 200 mM glucose-6-phosphate in a total volume of 1.0 ml. The reaction was started with an appropriate amount of enzyme protein. The mixture was incubated at 37°C for 30 min after which the reaction was stopped by addition of 1.0 ml of 10% TCA. The reaction mixture was centrifuged at 1000xg for 15 min and protein free supernatant was taken for the estimation of Pi according to the method of Taussky and Shorr (1953).

Glycogen Phosphorylase (α-1,4, Glucan : Orthophosphate Glucosyl Transferase; EC 2.4.1.1)

It was assayed according to the method of Rall et al. (1957). Assay mixture contained 0.2 ml mixture A [containing glycogen (57 mg); G-1-P (188 mg); NaF (42 mg) and 5'-AMP (4 mM) in 10.0 ml distilled water] and 0.1 ml B, enzyme protein (80-100 μg). Reaction mixture was incubated at 37°C for 30 min; the reaction was stopped by the addition 0.1 ml TCA (10 % w/v) and 0.4 ml sodium acetate (100 mM) was added to prevent spontaneous hydrolysis of G-1-P present in the incubation mixture. The reaction mixture was centrifuged at 1000xg for 15 min and protein free supernatant was taken for the estimation of Pi according to the method of Taussky and Shorr (1953).
Phosphofructokinase (ATP : Fructose-6-Phosphate-1-phosphotransferase; EC 2.7.1.11)

It was assayed according to the method of Racker (1947). The reaction mixture contained 200 mM tris-HCl buffer (pH 8.6), 10 mM MgCl₂, 10 mM F-6-P, 10 mM ATP, 0.24 mM NADH₂, 0.6 units aldolase, 17 units TPI, 2 units GDH and enzyme protein (250-300 μg). Optical density was measured at 340 nm for 3 min at 30 sec intervals.

Phosphoenolpyruvate Carboxykinase (GTP : Oxaloacetate Carboxylase (Phosphorylating); EC 4.1.1.32)

It was assayed according to the method of Ward et al., (1961). The reaction mixture contained 200 mM tris-HCl buffer (pH 7.4); 10 mM MnCl₂, 20 mM NaHCO₃, 1 mM GDP, 9 units MDH, 5 mM PEP, 0.24 mM NADH₂ and enzyme protein. Optical density was measured at 340 nm for 3 min at 30 sec intervals.

Pyruvate Kinase (ATP : Pyruvate Phosphotransferase; EC 2.7.1.40)

It was assayed according to Bucher and Pfleiderer (1955). The procedure was modified by using tris-HCl buffer in place of triethanolamine. The reaction mixture contained 200 mM tris-HCl buffer (pH 7.4), 100 mM KCl, 10 mM MgCl₂, 5 mM ADP, 5 mM PEP, 4 units LDH, 0.24 mM NADH₂ and enzyme protein. Optical density was measured at 340 nm for 3 min at 30 sec intervals.

Lactate Dehydrogenase (L-lactate : NAD OxidoReductase; EC 1.1.1.27)

Lactate dehydrogenase activity was determined according to the method of Kornberg (1955). The assay mixture contained 200 mM tris-HCl buffer (pH 7.4), 100 mM KCl, 5 mM sodium pyruvate and enzyme protein. The reaction was started by the addition of 0.24 mM NADH and the changes in absorbance were recorded at 340 nm for 3 min at 30 sec intervals.

Alcohol Dehydrogenase (EC 1.1.1.1)

Alcohol dehydrogenase was estimated according to the method of Agarwal and Goedde (1990). The reaction mixture contained 0.1 M Glycine-NaOH buffer (pH 9.6), 0.1 ml NAD (10 mg/kg), 0.1 ml ethanol (95 %). 0.1 ml of suitably diluted enzyme protein (post-mitochondrial fraction) were added and rise in optical density at 340 nm was recorded for 3 min at 30 sec intervals.
Protein Tyrosine Phosphatase (EC 3.1.3.48)

Protein tyrosine phosphatase activity was determined by modified method of Goldstein et al. (2000). Assay was performed in a final volume of 1.0 ml at 37°C for 30 min in reaction buffer containing 10 mM pNPP in 50 mM HEPES buffer (pH 7.0) with 1 mM DTT and 2 mM EDTA. The reaction was stopped by the addition of 500 µl of 0.1 M NaOH and the absorbance was determined at 410 nm. A molar extinction coefficient $1.78 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$ was utilized to calculate the concentration of the p-nitrophenolate ion produced in the reaction mixture.

Protein Estimation

Crude tissues homogenates were precipitated with an equal volume of 10 % TCA (w/v); washed twice with 5 % TCA and dissolved in 0.1 M NaOH. The total protein in serum and various tissues (liver, muscle, kidney) were estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Expression of Enzyme Activity

One unit of the enzyme is the amount required for the transformation of one mmol of substrate or the formation of one mmol of product per min under specified experimental conditions. Aspartate aminotransferase and alanine aminotransferase activities were expressed as µmol pyruvate formed/min/l serum. Activities of alkaline phosphatase and acid phosphatase were expressed as µmol p-nitrophenol released/min/dl serum. Activity of γ-glutamyl transpeptidase was expressed as µg p-nitroaniline released/min/dl serum whereas activity of sorbitol dehydrogenase was expressed as µ moles of NADH oxidized/min/dl serum. Activity of alcohol dehydrogenase in serum was expressed as µ moles of NAD reduced/min/dl serum. The extinction for oxidation or reduction of pyridine nucleotides was measured at 340 nm using silica cuvettes of 1 cm path length and extinction coefficient of $6.22 \times 10^6 \text{ cm}^2/\text{mole}$ (Horecker and Kornberg, 1948) was used to calculate reduced NAD/NADP. Glucose-6-phosphatase and glycogen phosphorylase activities were expressed as nmol Pi released/min/mg protein. Activities of phosphofructokinase, phosphoenolpyruvate carboxykinase, protein kinase and lactate dehydrogenase were expressed as nmol of NAD formed/min/mg of protein. Protein tyrosine phosphatase activity was expressed as nmol of p-nitrophenol formed/min/mg.
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Activity of alcohol dehydrogenase was expressed as nmol of NADH formed/min/mg protein.

Units of Biochemical/Hormonal Constituents

Blood Glucose values were expressed as mM and serum insulin and serum leptin were expressed as ng/ml. Serum C-peptide and GLP-1 were expressed as pM whereas serum adiponectin was expressed as μg/ml. Cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Apolipoproteins Apo A-II, Apo C-II, Apo E and bilirubin were expressed as mg/dl serum. Ethanol content was expressed as g/l. The level of total protein was expressed as mg/g tissue.

Statistical Analysis

Each biochemical parameter was expressed in terms of mean ± standard deviation. Groups were compared by analysis of variance with student 't' test. Significance difference were set at * p<0.05; ** p<0.01 or *** p<0.001.
RESULTS AND DISCUSSION

The diet intake of rats in both the groups was almost similar, however, the body weight gain in the case of the ethanol fed rats i.e. group II was less than that of the normal controls (group I) [data not shown].

Activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, acid phosphatase, $\gamma$-glutamyl transferase, sorbitol dehydrogenase and alcohol dehydrogenase were significantly higher in serum of alcohol-fed rats (Group II) compared to normal sucrose fed rats (Group I). The level of bilirubin was also increased in the serum of alcohol fed rats [Fig 2 (a-h)]. The fasting blood glucose level was significantly higher in alcoholic rats compared to control rats. Serum insulin level was also found to be significant higher in alcohol fed rats compared to sucrose fed rats. The serum levels of C-peptide and glucagon like peptide-1 (GLP-1) were also significantly increased after six weeks of alcohol feeding. The level of leptin in alcohol fed rats was also found to be increased compared to the rats of control group whereas the level of adiponectin in alcohol fed rats was observed significantly lower than sucrose fed rats [Fig 3 (a-f)]. The serum total cholesterol and triglyceride levels were significantly higher in alcohol-fed rats as compared with those of control animals. The levels of HDL-cholesterol and LDL-cholesterol were also found to be significantly higher in alcohol fed rats compared to sucrose fed rats. Apolipoprotein profiles (Apo A-II, Apo C-II and Apo E) were also observed significantly higher in the serum of alcohol-fed rats than the sucrose-fed rats. Alcohol fed rats had significantly higher level of ethanol in blood compared to control rats. [Fig 4 (a-h)].

Elevated serum aspartate aminotransferase and alanine aminotransferase in alcohol fed rats confirm the previous reports made on chronic alcoholism (Matloff, 1980). Elevation in other parameters of serum such as alkaline phosphatase, $\gamma$-glutamyl transpeptidase, sorbitol dehydrogenase and alcohol dehydrogenase confirmed the liver cell necrosis as evident in chronic alcoholics. It has been demonstrated that alcohol inhibits certain enzyme system in the liver. Administration of alcohol may reduce the activity of citric acid cycle in the liver. This may reduce the amount of available adenosine tri-phosphate and this inhibit the enzyme system dependent upon ATP, and extended for prolonged period which could result in cellular death.
Fig 2. Activities and Levels of Various Serum Parameters in Sucrose and Alcohol Fed Rats

a. Aspartate Aminotransferase

b. Alanine Aminotransferase

c. Alkaline Phosphatase

d. Acid Phosphatase

e. γ-Glutamyl Transpeptidase

f. Sorbitol Dehydrogenase

g. Alcohol Dehydrogenase

h. Bilirubin
Fig 3. Levels of Various Parameters in Sucrose and Alcohol Fed Rats

a. Blood Glucose

b. Insulin

c. C-Peptide

d. GLP-1

e. Leptin

f. Adiponectin

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Fig 4. Levels of Various Parameters in Serum of Sucrose and Alcohol Fed Rats

- a. Cholesterol
- b. Triglycerides
- c. HDL-Cholesterol
- d. LDL-Cholesterol
- e. APO A-II
- f. APO C-II
- g. APO E
- h. Ethanol
Ethanol is cleared from the body primarily by hepatic oxidation catalyzed initially by the NAD dependent cytoplasmic alcohol dehydrogenase to form acetaldehyde. Subsequently, the acetaldehyde is oxidized to acetate by mitochondrial aldehyde dehydrogenase. The resulting acetate is utilized in the mitochondrial Krebs cycle to provide energy. Continued oxidation of ethanol following chronic alcohol intoxication results in shifting of the intracellular redox state and decreased NAD to NADH ratio. Under such a condition, due to lack of NAD and abundant of NADH, the ethanol oxidation rate would be slowed and also the consequence of such redox state changes, fatty acid oxidation would be hindered thus causing fatty liver due to excess accumulation of triglycerides in the liver cells.

The present study showed the significant elevation in blood glucose levels in Charles Foster rats following alcohol feeding for 6 weeks. Serum insulin level was more prominent and significantly elevated in the alcoholic rats after 6 weeks of ethanol feeding. Plasma insulin levels were higher in these rats but plasma glucose levels remained high, suggest that the rats were resistant towards insulin. Increased insulin resistance and hyperinsulinemia in alcoholic rats might be linked to the development of hypertension as commonly evident in chronic alcoholics (Shinozaki et al., 1997; Kocemba et al., 1998).

Significant increase in the insulin level and impaired glucose tolerance in chronic alcohol fed rats might be due to insulin resistance caused by ethanol. After three weeks of ethanol feeding to Charles Foster rats, significant amount of deterioration occur in the oral glucose tolerance as compared to control group fed with standard diet and sucrose to balance isocaloric intake [Fig 5 (a-f)]. This development in the glucose intolerance becomes progressive every week deviating from the normal pattern of glucose tolerance. It clearly depicts the abnormality in oral glucose tolerance curve after six weeks feeding of 40 % ethanol. There is an increase in the fasting blood glucose levels as compared to normal rats, which reaches upto 8-10 mM after a glucose load of 2.5 g/kg body weight. The present study confirms that chronic ethanol feeding impairs glucose tolerance (Wilkes et al., 1996). Gluconeogenesis from glycogen, fatty acids, amino acids and lactate also impaired during ethanol metabolism. Thus, ethanol induced hyperglycemia or diabetes mellitus is due to hepatic and muscle insulin resistance and impairment of pancreatic endocrine system (Ishii and Ito, 1996).
Fig 5. Effect of Alcohol Feeding on Oral Glucose Tolerance Test (OGTT) in Charles Foster Rats

a. OGTT Before the Start of Sucrose & Alcohol Feeding (Day 0)

b. OGTT After Two Weeks of Sucrose & Alcohol Feeding (Day 14)

c. OGTT After Three weeks of Sucrose & Alcohol Feeding (Day 21)

d. OGTT After Four Weeks of Sucrose Alcohol Feeding (Day 28)

e. OGTT After Five Weeks of Sucrose & Alcohol Feeding (Day 35)

f. OGTT After Six Weeks of Sucrose & Alcohol Feeding (Day 42)
Chronic ethanol feeding impairs glucose tolerance; impaired glucose tolerance was associated with an inability to maintain plasma insulin levels (Kornhuber et al., 1990). Chronic alcoholism is frequently associated with impaired intermediary metabolism and insulin resistance. The cellular defects leading to insulin resistance have not been clearly defined but could result from reduced insulin binding or abnormalities in any one of several post-receptor steps. Chronic ethanol ingestion caused significant time dependent and selective changes in cell surface binding of insulin that was associated with subsequent post-receptor events. Hyperinsulinemia (which blocks lipolysis) is caused by a toxic effect of ethanol and its metabolites, independent of caloric input and over weight.

There is controversy regarding the effect of ethanol on glucose metabolism or insulin action. Epidemiological studies have shown that mild or moderate alcohol intake possibly be associated with increased insulin sensitivity (Rimm et al., 1995; Bell, 1996). However, some experimental data collected from studies of short duration or acute alcohol loading in humans or rodents indicate that ethanol administration leads to abnormal glucose homeostasis (Wilkes et al., 1996; Patel et al., 1991). In addition, ethanol acutely administered to humans reportedly caused acute insulin resistance (Shelmet et al., 1988; Avogaro et al., 1996). On the other hand, studies using cultured cells have shown acute alcohol exposures to impair insulin signal transduction (Xu et al., 1995; Bhavani et al., 1995). Epidemiological studies have suggested that alcohol consumption is an independent risk factor for the development of non-insulin dependent diabetes mellitus. Alcoholism is known to be associated with increased plasma levels of two novel diols, 2,3-butanediol and 1,2-propanediol metabolites known to impair insulin action in isolated adipocytes (Xu et al., 1998). Ethanol and acetaldehyde inhibited insulin-stimulated adipocyte lipogenesis and glucose oxidation in vitro at concentrations far greater than those found in alcoholic subjects, the two diols were extremely potent inhibitors of basal and insulin-stimulated adipocyte metabolism at concentrations far below those observed in alcoholic subjects. The diols are therefore potent inhibitors of basal and insulin-stimulated adipocyte metabolism. This effect may be relevant to the pathogenesis of insulin resistance in alcoholic subjects (Lomeo, 1988). Based on these previous reports, it is speculated that intake of even a moderate amount of ethanol might have health benefits including enhanced insulin sensitivity, while excessive ethanol...
consumption would induce insulin resistance. Hepatic glucose production was elevated with both acute- and chronic-ethanol administration, indicating impairment of insulin-induced suppression of hepatic glucose production. One of the study suggest that the insulin signaling step impaired by ethanol feeding is likely to be downstream from PI 3-kinase (Onishi et al., 2003). In the present study it is demonstrated that excessive consumption of ethanol is a factor leading to insulin resistance.

Several studies have demonstrated that chronic alcohol feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia and hypertriglyceridemia in a relatively short span of time in normal rats. Ethanol is a powerful inducer of hyperlipidemia in both animals and humans (Avogaro and Cazzolatu, 1975). Ethanol also causes changes in metabolism of lipoproteins. Chronic alcohol intake is known to produce hypercholesterolemia, hyperlipidemia and hypertriglyceridemia (Baraona et al., 1983; Baraona and Lieber, 1979). Previous studies have shown that the plasma cholesterol concentration increases with alcohol consumption (Goedde and Agarwal, 1981). The results of the present study also correlate with the above findings. The increase in serum triglycerides in alcohol fed rats may be due to decreased activity of lipoprotein lipase, which is involved in the uptake of triglycerides rich lipoproteins by extra-hepatic tissue. Concentration of total cholesterol was higher in patients with alcoholic abstinence syndrome as compared with control. The increase in content of cholesterol in high-density lipoproteins and of these lipoproteins was apparently due to stimulation of these substances formation and inhibition of their catabolism in liver impaired with alcohol (Triufanov et al., 1981). Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl esters from HDL to apo B-containing lipoproteins. Since alcoholics have high HDL-cholesterol, a defect in cholesteryl ester transfer could be responsible for the alcohol-induced alteration in cholesterol distribution between lipoproteins. This defective transfer of cholesteryl esters from HDL to LDL contributes to the high HDL-cholesterol levels in alcoholics (Savolainen, 1990). Comlekci et al., (2003) determined the serum leptin levels in patients with liver cirrhosis and found that patients with alcohol liver cirrhosis had higher leptin levels than in normal subjects. Our findings also support this fact in albino rats. Adiponectin is an adipocyte derived hormone, which has anti-inflammatory and insulin sensitising properties. The low level of adiponectin may be one of the causes of insulin resistance in alcohol fed rats. The glucagon like peptide 1 (GLP-1), is an important hormone from the gut that enhance nutrient-stimulated insulin
secretion. Our study shows that the level of GLP-1 is significantly increased in alcohol fed rats, which may help in the secretion of insulin and may be one of the causes of hyperinsulinemia in alcohol fed rats.

Content of lipids and apolipoproteins A-I, A-II and E were studied in blood plasma of patients with chronic alcoholism under conditions of alcoholic abstinence syndrome and remission. The increase in cholesterol content was accompanied by elevation in concentration of apo A-I, apo A-II, C-II. In the patients the content of apo E tended to decrease. Our findings in rats have the similar results except for apo E where the level increases after six weeks of alcohol feeding.

Effect of Ethanol Ingestion on Regulatory Enzymes of Carbohydrate Metabolism

Glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) are the major regulatory enzymes in gluconeogenesis pathway. As shown in Fig 6 (a,e) their activities get enhanced in the liver of alcohol fed rats compared to sucrose fed rats. Glucose-6-phosphatase activity was also found to be increased in the kidney and muscle of alcohol fed rats. PEPCK activity was observed to be increased in the kidney whereas found to be decreased in the muscle of alcohol fed rats. As shown in the Fig (6b) glycogen phosphorylase activity was found to be increased significantly in the liver of alcohol fed rats. The glycogen phosphorylase activity was also higher in the kidney and muscle of alcohol fed rats. Phosphofructokinase, one of the regulatory enzymes of glycolysis; its activity was found to be diminished in the tissues of alcohol fed rats (Fig 6 c). Pyruvate kinase plays an important role in the utilization of glucose and thereby helping glucose uptake. Its activity was observed to be decreased in the liver, kidney and muscle of alcohol fed rats (Fig 6d). The activity of lactate dehydrogenase (LDH) was also found to be decreased in the tissues of alcohol fed rats compared to rats of normal control group (Fig 6f). Protein tyrosine phosphatase activity was found to be modulated in alcohol fed rats especially in the insulin sensitive tissues like liver and muscle (Fig 6g). Its activity was found to be increased in the liver and muscle but no significance difference was observed in kidney following ethanol feeding to rats. This alteration in enzyme activities may be due to defect in carbohydrate metabolism possibly it is doing so by increasing the activities of glucose-6-phosphatase and PEPCK one of the main reasons of hyperglycemia. The alcohol dehydrogenase activity was decreased significantly in the liver of alcohol fed rats compared to normal control group whereas its activity was found to be slightly higher in the kidney and muscle of alcohol fed rats compared to sucrose fed rats (Fig 6h).
Fig 6. Activities of Various Enzymes of Carbohydrate Metabolism in Different Tissues in Sucrose and Alcohol Fed Rats

a. Glucose-6-Phosphatase

b. Glycogen Phosphorylase

c. Phosphofructokinase

d. Pyruvate Kinase

e. Phosphoenolpyruvate Carboxykinase

f. Lactate Dehydrogenase

g. Protein Tyrosine Phosphatase

h. Alcohol Dehydrogenase
The heterogeneous pathogenesis and progressive natural history of type 2 diabetes mellitus and insulin resistance contrive a formidable therapeutic challenge. Dual endocrine deficits of impaired insulin action and inadequate insulin secretion due to very high activity of protein tyrosine phosphatase in these animal models create an environment of chronic hyperglycemia and general metabolic disarray as evidenced from the activities of regulatory enzymes.

The elevated rate of gluconeogenesis is an important contributor to the severe hyperglycemia that develops in alcohol fed rats. The basal rates of glucose utilization are significantly reduced in alcohol fed rats; a defect attributed to a combination of reduced glucose transport and impaired flux through the rate-limiting enzymes of glycolysis; phosphofructokinase (Schaffer et al., 1986).

Phosphoenolpyruvate carboxykinase (PEPCK) is another enzyme, which controls the regulation of gluconeogenesis. Its activity was found to be increased in the liver of alcohol-fed rats. Another enzyme, glycogen phosphorylase, a rate limiting enzyme in glycogenolysis; its activity was found to be significantly increased in the liver of alcohol fed rats. Tyrosine phosphorylation of cellular proteins by protein kinases seems to play a profound but complicated role in β-cell growth, development and secretion. Protein tyrosine phosphorylation is controlled not only by tyrosine kinases but also by the activity of protein tyrosine phosphatases that dephosphorylate phosphotyrosine residues, an important step in signal transduction pathway in the insulin secretion/ action, which get disturbed in diabetes mellitus and insulin resistance. Protein tyrosine phosphatase, an enzyme of importance in insulin signaling pathway was found to be highly activated in the case of insulin resistance. Here also the activity of PTPase was observed to be significantly increased compared to normal rats in the muscle and to some extent in liver of alcohol fed rats. Liver alcohol dehydrogenase activity in cirrhotics was found to be considerably lower than in controls. Alcohol dehydrogenase activity was found to be decrease proportionately with the severity of liver disease in alcoholics (Patel et al., 1991). Our results also correlate with the above findings in albino rats.