activity of testis mitochondria was determined by measuring the percentage of [4-^{14}C]-cholesterol converted to [4-^{14}C]-pregnenolone and [4-^{14}C]-progesterone by the modified method of Robinson and Stevenson[8]. The total volume was 1.5 ml, which included 0.1 ml of 70 mM succinate used as the source of reducing equivalents. 2 mg mitochondrial protein and 20,000 cpm of [4-^{14}C]-cholesterol (50-60 mCi/mmole, New England Nuclear) were used in each assay. Oxygen consumption of testis mitochondria was determined by Warburg's manometric method, as has previously been described in detail[9]. The assays were initiated by the addition of 10 µl 1 M succinate. Protein was determined by the method of Lowry et al.[10], with bovine serum albumin used as standard. The significance of differences between means was tested by Student's test; p values < 0.05 were considered significant in the present study.

Results and discussion. Our findings are given in the table. Diabetes caused a body-weight loss of 51% ($p < 0.05$) and a testis weight loss of 44% ($p < 0.002$). Insulin treatment partially prevented these. Glycemia during diabetes was severely increased, and insulin normalized the diabetic state. Similar results were obtained by Paz and Homonnai[11]. Oxygen utilization was increased with succinate as substrate by 50% ($p < 0.002$) in the diabetic state, and insulin treatment completely prevented abnormal oxygen uptake. [4-^{14}C]-cholesterol conversion activity was severely inhibited ($p < 0.002$) and insulin treatment completely prevented it even to supranormal level. As this reaction is considered to be the rate-limiting step in steroidogenesis, this finding may be indicative of a reduction of testosterone production[12]. Coupled with this decrease in mitochondrial steroidogenesis there was an increase in the ATP synthesis rate by 39% ($p < 0.025$).

These observations suggest that the energy obtained from substrate oxidation can be diverted from ATP production to support steroidogenesis in testis mitochondria. In control animals the energy derived from succinate oxidation could be used for ATP synthesis, and this used to support other mitochondrial functions including reversed electron transport and the energy dependent transhydrogenase enzyme. If succinate supplies reducing equivalents for cholesterol conversion, it is probable that it would reduce NAD^+ by reversed electron transport, which then could reduce NAD^+P by the transhydrogenase enzyme[6,13]. However, in the diabetic state the reducing equivalents or energy that were potentially available to support mitochondrial steroidogenesis could now be used, at least in part, to enhance ATP synthesis. Similar observations, suggesting that energy obtained from succinate oxidation could be diverted from phosphorylation to support steroidogenesis, have been previously reported for ovarian mitochondria[14].

1 To whom reprint requests should be addressed.
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Association between apolipoproteins A-I and A-II as evidenced by immunochemical approach[1]

E.R. Briones and S.J.T. Mao

Atherosclerosis Research Unit, Mayo Clinic and Foundation, Rochester (Minnesota 55905, USA), 1 February 1982

Summary. Apolipoprotein A-I isolated from human plasma high density lipoproteins were studied for its possible association with ^{125}I-labeled A-II. Using immunoprecipitation technique, we found that A-II, but not C-II and C-III, associated with A-I. The association was completed within 30 min and was temperature dependent.

Apoproteins A-I and A-II are the Major protein moieties of human plasma high density lipoproteins (HDL). The apoproteins are synthesized in the liver and intestine[2,3]. The mechanism involved in the assembly of apoA-I and apoA-II into HDL in vivo is still not clear. The molecular and solution properties of the apoproteins have recently been reviewed by Osborne and Brewer[4]. The proteins tend to self-associate and form aggregates in aqueous solution. The degree of self-association is dependent on the concentration[4] and on the temperature[5]. However, whether or not this apoprotein exists as an equilibrium mixture of monomers and dimers, dimers and tetramers, or monomers, dimers, tetramers and octamers is still controversial[4-9]. Besides self-association, these two apoproteins may associate with each other and form a hybrid. Using a cross-linking reagent, it has shown some degree of interaction between apoA-I and apoA-II[5]. Whether or not the association is temperature dependent is not known. In addition, the ability of apoA-I to associate with apoproteins C has not been reported. Alternatively, we now report the association between apoA-I and apoA-II using an immunochemical method. The association between apoA-I and ^{125}I-labeled apoC-II and apoC-III was also investigated. Understanding of the protein-protein interaction of these apoproteins may help us to elucidate the molecular orientation of the apoproteins in HDL as well as their metabolic pathway in vivo.

Materials and methods. ApoA-I and apoA-II were isolated by gel filtration on Sephadex G-150 in the presence of 5 M guanidine HCl, pH 8.0[10]. Homogeneity of apoproteins was determined by polyacrylamide gel electrophoresis and by amino acid analysis. Goat and rabbit anti-apoA-I antisera were obtained using the procedures described previously[10-13]. The antisera were then partially purified by

$(NH_4)_2SO_4$ fractionation. ApoA-I and apoA-II were iodinated with Na ^{125}I using a modification of the chloramine-T method[10]. Final specific activities of ^{125}I-labeled apoA-I and apoA-II were approximately 10 μCi/μg and 12 μCi/μg, respectively. Radiolabeled apoA-II showed 1 single peak on G-150 column chromatography in the presence of 5 M guanidine. Double antibody radioimmunoassays were used to determine the specificity of the anti-apoA-I antibodies[10]. The anti-apoA-I did not form precipitin lines against apoA-II by an immunodiffusion technique. To study the mixed association of apoA-I and apoA-II, we used the following immunoprecipitation technique: 1. apoA-I (20 μg) in 100 μl buffer solution containing 0.01 M Tris, 0.01 M NaCl, and 0.001 M EDTA, pH 7.4, was preincubated with 100 μl of ^{125}I-labeled apoA-II containing approximately 16,500 cpm. The reaction was allowed to proceed for 2 h at 37 °C; 2. undiluted goat anti-apoA-I (60 μl) were added to the reaction mixtures followed by another incubation at 37 °C for 4 h; and 3. buffer solution (2 ml) containing 0.1% BSA was then added to wash the immunoprecipitate and the tubes centrifuged at 3000 rev./min (1000 × g, Beckman Model TJ-6 centrifuge) for 40 min. The supernatant was aspirated and the precipitate was counted in an Autogamma counter.

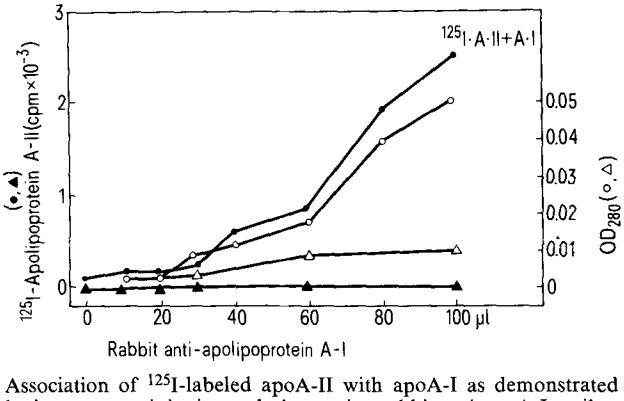
Results. The purpose of the present study was to examine the possible association between apoA-I and apoA-II, apoA-I and apoC-II, and apoA-I and apoC-III in an aqueous solution. The strategy was to utilize specific antibodies to precipitate apoA-I and to 'co-precipitate' ^{125}I-labeled apoproteins if there was any association. To determine the specificity of anti-apoA-I antibodies, increasing amounts of goat or rabbit anti-apoA-I were incubated with ^{125}I-labeled apoA-I or apoA-II. The antibodies were specifically bound to labeled apoA-I but not apoA-II. Standard displacement curves were performed. Only unlabeled apoA-I, but not apoA-II, apoC-II, and apoC-III, was able to displace ^{125}I-labeled apoA-I from the antibodies. Using rabbit anti-apoA-I antibodies, we show the association between apoA-I and ^{125}I-labeled apoA-II (fig.). ^{125}I-labeled apoA-II in the presence of unlabeled apoA-II (20 μg) was not precipitated by rabbit anti-apoA-I antibodies. However, when apoA-I (20 μg) was added, the ^{125}I-labeled apoA-II was found to be associated with the immune complex. Since the rabbit anti-apoA-I was specific[10], it appears that there was an association between apoA-I and labeled apoA-II. The result was reproducible when goat anti-apoA-I was utilized. We have also examined the possible association of apoA-I with labeled apo-C-II and apoC-III. Identical procedures were used for this study. No association was seen (table). A time course experiment was done in which apoA-I (20 μg) were incubated with ^{125}I-labeled apoA-II (1 ng) for various lengths of time. The goat anti-apoA-I (60 μl), which produced maximal immunoprecipitation, were added to all the tubes after the different incubation periods at 37 °C. Maximal association of apoA-I with apoA-II by this technique was observed at approximately 30 min. To determine if the association of apoA-I and apoA-II was altered by changes in the temperature, 3 different temperatures, 4, 37, and 24 °C, were chosen for comparison. The ideal temperature for association was at 24 °C. A slight, but not significant, decrease in binding was seen at 37 °C. Binding was lowest at 4 °C and at that temperature only 62% of that seen at 24 °C.

Discussion. High density lipoproteins have recently become an important subject because of the negative correlation between HDL cholesterol levels and the development of coronary heart disease[14]. The molecular orientation of the major apoproteins A-I and A-II in the HDL particles is not well known. The interaction between apoproteins A-I, A-II, C-II, and C-III in HDL particles is not known. Using dimethylsuberimidate as a cross-linking reagent, Swaney and O'Brien[5,15] have shown the hybridization of apoA-I with apoA-II in a mixed solution. The major disadvantage of using a cross-linking reagent is that the association may be produced in part by the reagent. The immunoprecipitation technique used in the present study appears to have some advantages: 1. it is simple and specific; 2. it supports the direct evidence of apoprotein association; and 3. using ^{125}I-labeled apoA-II, the antibodies are able to separate apoA-II associated with apoA-I. Apparently, the association between apoA-I and apoA-II was very rapid. The maximal association was completed within 30 min. The actual time required for association was difficult to assess since the antibodies added to the mixtures of apoA-I and apoA-II may not inhibit or stop the hybridization. In one case, we have incubated anti-apoA-I with apoA-I for 24 h prior to the addition of ^{125}I-labeled apoA-II. The antibodies inhibited only 40% of the hybridization (not shown). The inhibition was probably due to the denaturation and precipitation of apoA-I by the antibodies. It was unlikely that the association of apoA-II to apoA-I would result in a decrease of the reaction of anti-apoA-I and apoA-I. Hence, an increasing amount of apoA-II did not inhibit the ^{125}I-labeled apoA-I bound to its antibodies. On the other hand, it was not certain that the apoA-I antibodies would lower the affinity (Ka) between apoA-I and apoA-II. A recent study[5] has demonstrated that there was no self-association of apoA-I or apoA-II at temperatures above 30 °C. Our result shows that there was no significant

Specificity of the association of ^{125}I-labeled apoA-II, apoC-II, and apoC-III with apoA-I as determined by an immunoprecipitation technique[a]

	Control	Sample tested		
	A-I	A-II	C-II	C-III
Specific radioactivity (μCi/μg)	~10	~12	~10	~10
Amount added in cpm	14,873	16,607	28,000	23,826
Associated with A-I	10,068	6,742	293	335
Nonspecific binding	900	872	149	293

[a] 20 μg of apoA-I in 100 μl buffer solution was incubated with 100 μl of ^{125}I-labeled apolipoproteins A-I, A-II, C-II, and C-III at 37 °C for 2 h. Goat anti-apoA-I (60 μl) was then added and the mixture incubated at 37 °C for 4 h. The immunoprecipitate was washed and counted as described in the text. Nonspecific binding was evaluated in the absence of goat antibodies.



Association of ^{125}I-labeled apoA-II with apoA-I as demonstrated by immunoprecipitation technique using rabbit anti-apoA-I antibodies. ^{125}I-labeled apoA-II (~ 16,500 cpm) was incubated with 20 μg of apoA-II (▲) or 20 μg of apoA-I (●) before the addition of antibodies. Immunoprecipitate (if any) of apoA-II (△) or apoA-I (○) was dissolved in 2 ml of 0.1 N NaOH and read at 280 nm.

difference for hybridization between the incubation at 24 and 37 °C. This suggests that self-association probably does not play an important role in apoA-I and apoA-II hybridization. It is of interest that the apoA-I did not associate with ^{125}I-labeled apoC-II and apoC-III. The apo C proteins in plasma are in equilibrium between HDL and triglyceride-rich particles. In vitro, the C proteins exchange rapidly between triglyceride-rich particles and HDL[16].
In vivo there is a net transfer of C proteins from HDL to triglyceride-rich particles but no transfer of A proteins[17] following a meal. Our finding that apoproteins C-II and C-III did not directly associate with apoprotein A-I suggests that apoprotein A-I in HDL may not play a direct role for the net exchange of apoproteins C between HDL and triglyceride-rich particles. Furthermore, it would be of interest to utilize anti-apoA-II antibodies as a reverse experiment to demonstrate if ^{125}I-labeled apoA-I would associate with apoA-II.

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Effect of 5-thio-D-glucose on blood glucose and glucose-6-phosphatase activity in mice

K. Veeraragavan, P. Rathinasamy and S. Ramakrishnan[1]

Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-605006 (India), 14 August 1981

Summary. Blood glucose was significantly elevated by 5-thio-D-glucose administration (25, 50, and 100 mg/kg). The rate-limiting enzyme glucose-6-phosphatase of liver was also increased. The elevation of blood glucose was due to the rapid glycogenolysis of liver.

5-Thio-D-glucose[2], a structural analogue of D-glucose causes an increase of blood glucose when it is administered i.p., i.v. and also p.o.[3,4]. The compound inhibits glucose uptake and glycolysis in liver, kidney and diaphragm[5]. The increase in blood glucose concentration from liver and kidney was believed to be controlled through glucose-6-phosphatase activity[6]. Since the compound increases blood glucose, the glucose-6-phosphatase activity was studied in intestines, liver and kidneys.

Materials and methods. Male albino mice weighing 25–30 g were used. The animals were maintained on the stock laboratory diet (obtained from Hindustan Lever, India) and water ad libitum. Glucose-6-phosphate (disodium salt) was obtained from Sigma Chemicals Co. (St. Louis, USA) and glucose oxidase from Koch-Light Laboratories Ltd (England). All animals were fasted for 24 h before the experiment and after a single gastric intubation of 5-thio-D-glucose (25, 50 and 100 mg/kg b.wt) were sacrificed by decapitation at various intervals (0, 30, 60, 90, 120, 150 and 180 min) for samples of blood and tissues, viz., liver, kidneys and intestines. Blood glucose was estimated by the method of Huggett and Nixon[7] as adopted by Krebs et al.[8]. Microsomes were prepared from the homogenates of liver, kidneys and intestines according to the method of Jorgensen[9] as described by Suketa et al.[10]. Glucose-6-phosphatase activity was estimated in the heavy microsomal fraction obtained by centrifugation (25,000 × g, 30 min) of the supernatant and sedimentation of the mitochondria at 10,800 × g for 30 min. All the preparations were carried out at 4 °C. The glucose-6-phosphatase activity was determined by the method of Yeung et al.[11]. The activity was expressed as units/g tissue. 1 unit of the enzyme releases 1 µmole of inorganic phosphate per min from glucose-6-phosphate at 30 °C.

Results and discussion. With graded doses of 5-thio-D-glucose (5 TG), i.e., 25, 50 and 100 mg/kg b.wt, there was an increase in blood glucose with time (fig. 1). With all the concentrations of 5 TG, blood glucose reached a maximum in 30 min and returned to near normal within 180 min. It was observed that the rise in blood glucose was proportional to the dose of 5 TG. The blood glucose was maximum in 30 min sample and then slowly decreased to almost normal in 180 min sample.

A continuous source of blood glucose is the hydrolysis in liver, kidneys and intestines of glucose-6-phosphate, which is derived either from glycogenolysis or other potential precursors[12]. Gluconeogenesis in liver[13] and kidney cortex[8], is well documented and both the tissues contain the enzymes of gluconeogenesis[14]. Hydrolysis of glucose-6-phosphate to glucose is effected by glucose-6-phosphatase and