Apolipoprotein A_1 Expression in Young and Aged Rats Is Modulated by Dietary Carbohydrates

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To determine if dietary carbohydrates modulate apolipoprotein A_1 ($ApoA_1$) expression, plasma $ApoA_1$ protein and hepatic $ApoA_1$ mRNA levels were measured in young and aged rats maintained on a high-fructose (60% of diet weight consisting of fructose), or high-glucose (60% glucose) diet or fed regular rat chow for 10 days. Aged rats on regular chow had significantly higher plasma $ApoA_1$ concentrations and hepatic $ApoA_1$ mRNA than young rats maintained on this diet. Plasma $ApoA_1$ and hepatic $ApoA_1$ mRNA levels in young rats or aged rats maintained on the 60% fructose diet were significantly higher than in rats within the same age group maintained on regular rat chow ($P < .01$). Similar induction of $ApoA_1$ protein and mRNA was found in rats maintained on the 60% glucose diet ($P < .01$). It is concluded that $ApoA_1$ expression in rats is modulated by factors related to the nature of dietary carbohydrates rather than insulin resistance associated with high-fructose feeding.

DIETARY FACTORS are known to modulate hepatic gene expression.[1] Some of the effects are related to insulin secretion, whereas others are independent of ambient insulin concentrations.[2] Although insulin is known to modulate high-density lipoprotein (HDL) cholesterol levels, it is not well established whether the low HDL cholesterol concentrations in hyperinsulinemic syndromes are the direct result of insulin resistance or secondary to another biochemical event commonly found in these subjects.[3,4] To address this issue, we studied apolipoprotein A_1 ($ApoA_1$) expression in experimental insulin resistance induced by a high-fructose diet compared with similar diets in which fructose was substituted with glucose (high-glucose diet) or a complex carbohydrate such as corn starch (regular rat chow). Since aging is often associated with an altered response to dietary and hormonal changes, we also studied $ApoA_1$ expression in aged rats maintained on either the high-fructose or high-glucose diet or regular rat chow. The results indicate that the high-fructose diet and high-glucose diet are associated with increased expression of $ApoA_1$.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats were obtained from the National Institute of Aging Colony maintained by Harlan Laboratories (Indianapolis, IN). Three- to 4-month-old (young) rats were compared with 25-month-old (aged) rats. After 1 week at our animal facility, rats at each age group were divided into the following dietary groups: control diet, standard rat chow with approximately 62% of calories as complex carbohydrates, 23% as protein, and 14.9% as fat; high-glucose diet, with 65.7% of calories as dextrose (60% of its weight as dextrose), 21.9% protein, and 12.3% fat; and high-fructose diet, with 65.7% of calories as fructose (60% of its weight as fructose), 21.9% protein, and 12.3% fat. All rats were maintained on this diet and had water ad libitum for 10 days. Body weight and food intake were measured every other day during the study.

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The rats were killed by exsanguination through the abdominal aorta under sodium pentobarbital anesthesia (45 mg/kg intraperitoneally), and hepatic tissue was collected and immediately frozen in liquid nitrogen.

Internal organs of all the animals were inspected for gross pathology. Animals bearing tumors were removed from the analysis.

Plasma glucose levels on the day of death were measured with a glucose oxidase technique using the Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin level was measured by a commercial rat specific insulin radioimmunoassay kit (Linco Research, St Charles, MO).

$ApoA_1$ Purification

Purification of the rat $ApoA_1$ was performed to make suitable antibodies for Western blot analysis. First, HDL was purified from rat serum following a procedure adapted from Jensen et al.[5] Solid KBr (345 mg) was added to 1.5 mL rat serum to achieve a density of approximately 1.175 g/mL, and the serum was placed in a Beckman ultraclear centrifuge tube fitted for the Beckman SW41 rotor. KBr solutions (2.3 mL) with densities of 1.15, 1.125, 1.100, and 1.075 g/mL, respectively, and, finally, 0.5 mL KBr solution with a density of 1.05 g/mL were overlaid slowly using a pipette. The tubes were centrifuged at 284,000 *g* (40,700 rpm) at 15°C for 22 hours in a Beckman L8-80 ultracentrifuge. Fractions containing HDL were collected by puncturing the centrifuge tube approximately 1 cm from the bottom with a 20-gauge needle attached to a peristaltic pump operating at about 1 mL/min. Fractions were pooled and dialyzed against 0.9% NaCl and identified as containing HDL/$ApoA_1$ by polyacrylamide gel electrophoresis (PAGE).[6]

Protein in the HDL/$ApoA_1$-containing fraction was acetone-precipitated by adding 10 vol acetone and cooling at −20°C for 30 minutes. The protein was then precipitated in a 50-mL polypropylene tube at 12,000 rpm for 15 minutes using a JA 20 rotor and a Beckman J-21C centrifuge. After removal of the acetone, the protein was resuspended in 0.3 mL sodium dodecyl sulfate (SDS) loading buffer.[7] Purification of $ApoA_1$ was then performed using the Model 491 BioRad (Hercules, CA) Prep Cell by running a 6-cm 9.5% analytical gel for approximately 4 hours; 2.5-mL fractions were monitored for $ApoA_1$ by SDS-PAGE and silver staining, and then pooled and concentrated to a volume of about 1 mL using a centriprep-10 concentrator (Amicon, Beverly, MA). Next, glycine and SDS were removed by first dialyzing against 0.9% NaCl and then precipitating with 10 vol acetone. Pure $ApoA_1$ was resuspended in 0.9% NaCl and dialyzed once more against 0.9% NaCl to remove any traces of SDS. The authenticity of rat $ApoA_1$ was validated by molecular size and by amino acid analysis of the purified protein excised directly from the gel, which showed 93% homology with the predicted amino acid composition.

Preparation of Rat $ApoA_1$ Antibody

Purified $ApoA_1$ protein (20 μg dissolved in 3 mL 0.9% saline) was resuspended in Freund adjuvant (Sigma, St Louis, MO) and injected subcutaneously on the backs of two New Zealand rabbits. The first test of antibody specificity at 6 weeks showed a very weak signal using the Amersham immunoblotting kit (Amersham, Arlington Heights, IL). The rabbits were reinjected with another dose of $ApoA_1$ protein (20 μg) divided between the two rabbits. Serum from the rabbits was retested at 12 weeks from the initial injection and found to be of sufficient strength to be used in these studies. A third injection totaling 14.1 μg divided between the two rabbits was administered 3 months following the initial injection, and the serum was tested at 4 months from the time of initial injection. Serum from the latter injection was used at a 1:10,000 dilution for Western blots.

Measurements of Plasma $ApoA_1$

Plasma proteins (25 μg) from 4- and 25-month-old rats maintained on various test diets were electrophoresed in denaturing SDS, 12% polyacrylamide gel under reducing conditions.[6] Proteins in the gels were electrophoretically transferred to a nitrocellulose membrane.[7] The membrane was incubated with $ApoA_1$ antibody at a final dilution of 1:10,000 for 2 hours at room temperature. Horseradish peroxidase–linked goat anti-rabbit IgG was used at a final dilution of 1:10,000 for 1 hour at room temperature. Blots were developed using an enhanced chemiluminescence (ECL) Western blotting technique (ECL Kit) as described by the manufacturer (Amersham). Plasma $ApoA_1$ was determined by densitometry using the personal densitometer from Molecular Dynamics (Sunnyvale, CA). The summed absorbance of bands was analyzed after subtraction for background. Reproducibility of $ApoA_1$ quantitation was established with gels loaded with different amounts of authentic rat $ApoA_1$.[8,9] The correlation coefficient between the amount of rat $ApoA_1$ applied to the gel and the optical density of the band found on immunoblots was .99 (Fig 1). Interassay and intraassay coefficients of variation for the measurements were 12.8% and 6.5%, respectively.[9]

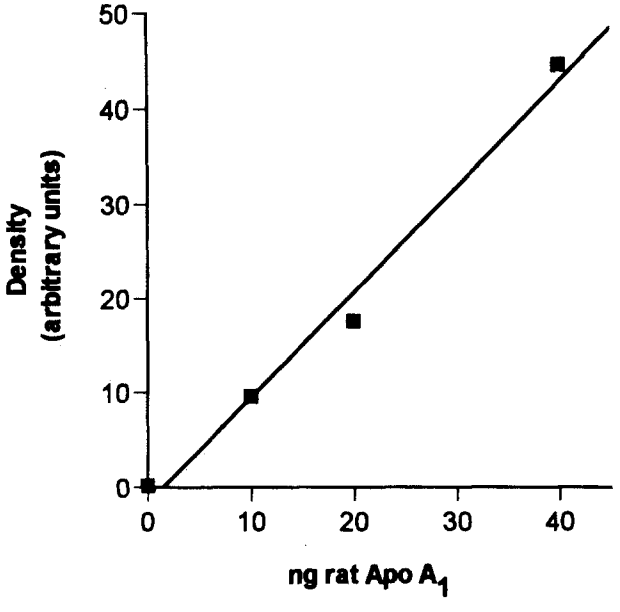

Fig 1. Linear correlation between the amount of purified rat $ApoA_1$ applied to the gel and the measured optical density of $ApoA_1$ detected on immunoblots (r = .99).

Table 1. Body Weight on the Day of the Experiment, Daily Food Intake, and Plasma Glucose and Insulin Concentrations of Rats on the Three Diets

Diet Group	Body Weight (g)	Food Intake (g/rat/d)	Plasma Glucose (mg/dL)	Plasma Insulin (μU/mL)
Rat chow				
3.5 mo	304.6 ± 5.2	18.9 ± 0.7	136.3 ± 3.5	91.9 ± 7.9
25 mo	449.0 ± 6.3*	14.1 ± 0.8*	132.1 ± 3.8	44.7 ± 6.4*
60% glucose				
3.5 mo	309.2 ± 8.3	19.8 ± 0.9	132.0 ± 3.1	68.8 ± 11.7
25 mo	447.2 ± 5.3*	17.2 ± 1.0†	131.3 ± 3.4	67.6 ± 9.6
60% fructose				
3.5 mo	283.6 ± 4.4	15.8 ± 0.9†	176.0 ± 4.3†	132.3 ± 7.7†
25 mo	454.3 ± 5.9*	12.9 ± 1.0	135.0 ± 8.0*	55.5 ± 10.6*

NOTE. Results are the mean ± SEM; n = 10 in each group.
*$P < .01$ v 3.5 mo within each diet group.
†$P < .01$ v rat chow diet within each age group.

Measurements of Hepatic $ApoA_1$ mRNA Content

Livers from 4- and 25-month-old rats were immersed in liquid nitrogen and stored at −70°C. RNA in these livers was isolated by cesium chloride density gradient centrifugation.[10] Recombinant plasmid pBR322 containing rat $ApoA_1$ cDNA (NE-477) was kindly provided to us by Dr J.I. Gorden of Washington University (St Louis, MO). Amplification and preparation of plasmid DNA was accomplished by established procedures.[11] The 412-bp NE-477 insert was cleaved from the purified recombinant plasmid DNA by *Eco*RI and *Sac*I using conditions specified by the supplier. The cleaved insert was separated from the vector DNA and random primer–labeled with ^{32}P-dCTP.[12] The isolated RNA was electrophoresed in 1.5% agarose gels in the presence of 2.2 mol/L formaldehyde,[13] transferred to the nylon membrane by diffusion blotting, and finally hybridized with the $ApoA_1$ cDNA insert.[14] The membrane was exposed to x-ray film for autoradiography. The blots were stripped and reprobed with G_3PDH cDNA to establish the specificity of age-related changes in $ApoA_1$ mRNA.

All results are expressed as the mean ± SEM. Two-way ANOVA followed by Tukey's test was performed to establish statistical significance of the differences.

RESULTS

Body weight on the day of the experiment, daily food intake, and plasma glucose and insulin concentrations are summarized in Table 1. Some of these data have also been included in previously published reports.[8,15] As expected, older rats weighed more and consumed fewer calories when given regular rat chow. However, there were no age-related differences in food consumption when rats were given a high-glucose (60%) or high-fructose (60%) diet. Plasma glucose and insulin concentrations were significantly elevated in young rats maintained on a high-fructose diet, indicating that an insulin-resistant state induced by the high-fructose diet had occurred in this group. However, aged rats maintained on a high-fructose diet did not have significantly altered plasma glucose or insulin levels. In general, plasma insulin concentrations measured in the morning in a nonfasting state tended to be lower in aged rats compared with young controls.

A representative Western blot of plasma proteins is shown in Fig 2. The expected single 28-kd band of $ApoA_1$ is visible. When the antiserum preabsorbed with purified $ApoA_1$ was used, no bands could be seen on immunoblots, indicating the

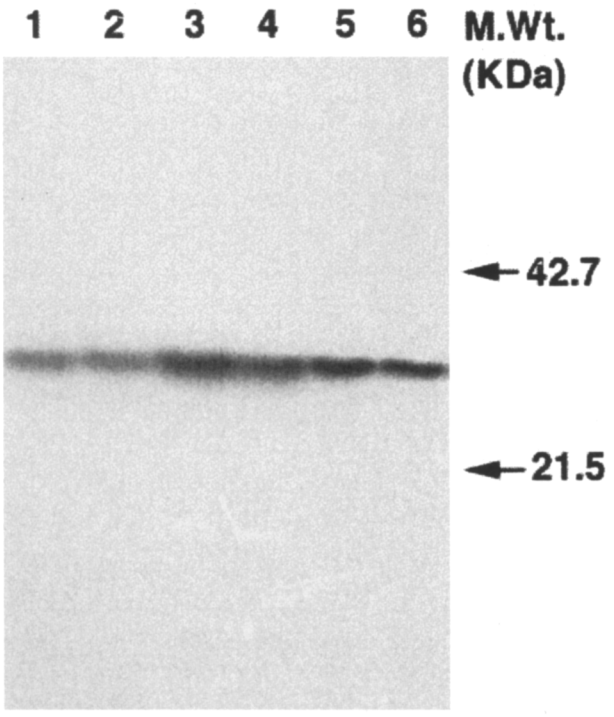

Fig 2. Representative Western blot of plasma proteins (25 μg) from young rats on regular chow (lanes 1 and 2), 60% fructose diet (lanes 3 and 4), or 60% glucose diet (lanes 5 and 6). $ApoA_1$ was probed with rabbit anti-rat $ApoA_1$ antiserum at 1:10,000 dilution for 2 hours and developed with an ECL technique. An expected band at 28 kd is seen.

specificity of the antiserum used. The mean ± SEM for plasma $ApoA_1$ estimates from various immunoblots in arbitrary units are summarized in Fig 3. Aged rats on regular chow or a 60% glucose diet had significantly higher plasma $ApoA_1$ concentrations than young rats maintained on these respective diets ($P < .01$). Plasma $ApoA_1$ in young rats maintained on 60% fructose (167.1 ± 16.3) or 60% glucose (155.4 ± 26.3) was significantly higher than in young rats maintained on regular rat chow (61.3 ± 12.4, $P < .01$). Similarly, in aged rats, those maintained on 60% fructose (224.0 ± 57.8) or 60% glucose (225.4 ± 26.1) had higher plasma $ApoA_1$ concentrations than aged rats on regular rat chow (96.3 ± 12.6, $P < .01$).

To assess whether the increased abundance of plasma $ApoA_1$ was paralleled by a corresponding increase in the mRNA, we measured the hepatic content of $ApoA_1$ mRNA by Northern blot analysis. A representative Northern blot containing total hepatic RNA hybridized with $ApoA_1$ cDNA and G_3PDH cDNA is shown in Fig 4. The expected 0.9-kb band of $ApoA_1$ mRNA and the corresponding 1.2-kb band of G_3PDH are visible. The mean ± SEM for $ApoA_1$ mRNA estimates from various Northern gels in arbitrary units are summarized in Fig 5. Hepatic $ApoA_1$ mRNA content of young rats maintained on 60% fructose (1.13 ± 0.08) or 60% glucose (1.49 ± 0.17) was significantly greater than in young rats on regular chow (0.69 ± 0.07, $P < .01$). Aged rats maintained on a high-fructose diet had a significantly increased hepatic $ApoA_1$ mRNA level compared with controls (2.03 ± 0.19 *v* 1.38 ± 0.13, $P < .01$). The increase in $ApoA_1$ mRNA in rats on a high-glucose diet (1.77 ± 0.17) did not reach statistical significance. Overall, aged rats on regular chow or a high-fructose diet had higher hepatic $ApoA_1$ mRNA content compared with young rats on either of the two test diets ($P < .01$). The difference between young and aged rats maintained on a high-glucose diet did not reach statistical significance.

DISCUSSION

The results clearly show that aged rats have higher plasma $ApoA_1$ along with increased hepatic $ApoA_1$ mRNA content. This is in agreement with previously published studies.[8,15] In general, the increase in plasma $ApoA_1$ with dietary changes or age was accompanied by an increase in hepatic $ApoA_1$ mRNA, suggesting that expression of $ApoA_1$ is regulated at a pretranslational level. Alternatively, it is possible that a parallel decrease in $ApoA_1$ protein and mRNA clearance with age may account for the findings. The latter speculation is supported by recent data on $ApoA_1$ expression in aging rats.[8]

Both plasma $ApoA_1$ concentration and hepatic $ApoA_1$ mRNA content were increased during high-fructose feeding in both young and aged rats. This change appeared to be independent of the insulin-resistant state, since high-glucose feeding, which does not cause significant insulin resistance, was also associated with increased $ApoA_1$ concentrations. In addition, the degree of increase over baseline in plasma $ApoA_1$ of aged rats on a high-fructose diet (2.3-fold) was not significantly different from that of young rats (2.7-fold), even though aged animals tend to have more insulin resistance at basal dietary conditions.[16] These data indicate that insulin resistance in rats occurring as a result of dietary change is associated with an increased $ApoA_1$ concentration rather than a reduced $ApoA_1$ expression. However, the mechanisms of $ApoA_1$-inducing effects of high-

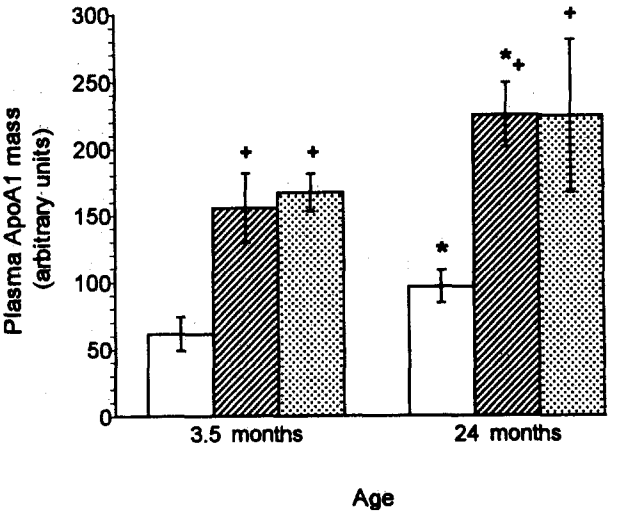

Fig 3. Mean ± SE optical density in arbitrary units of $ApoA_1$ bands identified on various immunoblots of plasma proteins. □, regular chow; ▨, 60% glucose diet; ▩, 60% fructose diet. *$P < .01$ *v* 3.5-month-old rats within each diet group. †$P < .01$ *v* rats on regular rat chow within each age group. n = 10 per group.

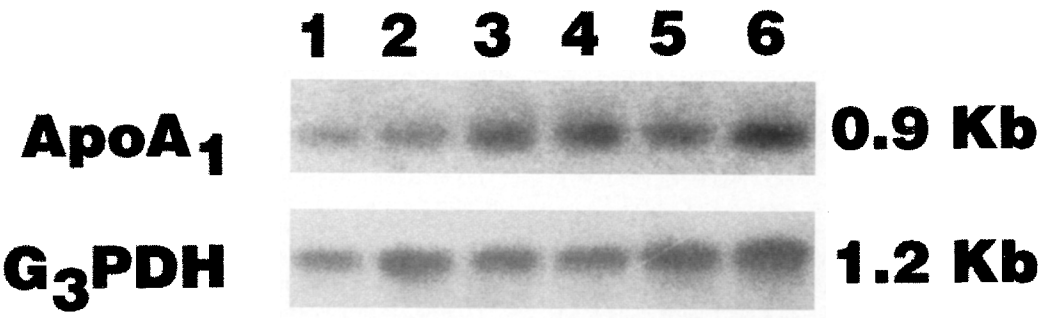

Fig 4. Representative Northern blot of rat hepatic RNA hybridized with $ApoA_1$ cDNA and G_3PDH cDNA. Lanes 1 and 2, young rats on regular chow; lanes 3 and 4, young rats on 60% fructose diet; lanes 5 and 6, young rats on 60% glucose diet. A 0.9-kb band of $ApoA_1$ mRNA and 1.2-kb band of G_3PDH mRNA are visible.

glucose or high-fructose diets are not clear. It is tempting to speculate that an intracellular metabolic signal is generated in response to high ambient concentrations of glucose or fructose in the portal circulation, that leads to upregulation of $ApoA_1$ expression.

It is noteworthy that an insulin-resistant state induced by high-fructose feeding could be documented only in young rats by measurement of increased plasma glucose or insulin concentrations (Table 1). These changes could not be documented in aged rats. The discrepancy is probably a result of the plasma collection for glucose and insulin measurements being made in the nonfasting state and therefore unstandardized as to food intake. This may explain why plasma insulin in aged rats on regular chow or high-fructose feeding was lower than in young rats. It is possible that the reduced food intake of aged rats maintained on regular chow or high-fructose meal blunted the expected postprandial hyperinsulinemia. Another caveat is that intestinal $ApoA_1$ mRNA content was not measured in this study. It is estimated that in rats up to 55.7% of plasma $ApoA_1$ is synthesized in the intestine.[17] Thus, the contribution of changes in intestinal $ApoA_1$ mRNA to the increase in plasma $ApoA_1$ following high-fructose or high-glucose feeding is not known. It is tempting to speculate that these changes are related to factors local to the intestine where the metabolism of simple carbohydrates such as glucose or fructose differs from that of complex carbohydrates in the regular chow. Thus, it is possible that such local factors may override the systemic effects of insulin resistance on hepatic $ApoA_1$ synthesis.

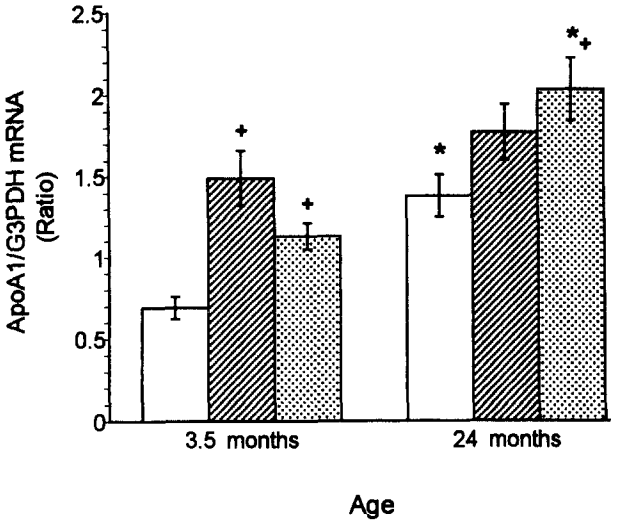

Fig 5. Mean ± SE hepatic $ApoA_1$ mRNA (in arbitrary units) from young and aged rats maintained on regular chow (□), 60% glucose diet (▨), or 60% fructose diet (▩). *$P < .01$ *v* 3.5-month-old rats within each diet group. +$P < .01$ *v* rats on regular chow within each age group.

It is noteworthy that fructose feeding of non-obese rats such as the Fischer 344 strain may not be the most appropriate model to study hyperinsulinemia. Similar studies in Wistar rats should be made.

Overall, it appears that high-fructose or high-glucose feeding is associated with increased $ApoA_1$ levels. The high-fructose diet induced $ApoA_1$ expression in aged rats without apparent hyperinsulinemia. Thus, in the rat $ApoA_1$ expression is modulated by factors related to the nature of dietary carbohydrates rather than insulin resistance associated with high-fructose feeding. Future studies with different experimental models of insulin resistance should be made to ascertain the pathogenetic link between insulin and low HDL in clinical insulin resistance syndromes.

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