Adaptation of intestinal production of apolipoprotein A-IV during chronic feeding of lipid

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Kalogeris, Theodore J., and Richard G. Painter. Adaptation of intestinal production of apolipoprotein A-IV during chronic feeding of lipid. Am J Physiol Regulatory Integrative Comp Physiol 280: R1155–R1161, 2001.—We examined the effect of daily fat supplementation on intestinal gene expression and protein synthesis and plasma levels of apolipoprotein A-IV (apo A-IV). Rats were fasted overnight and then given intragastric bolus infusion of either saline or fat emulsion after 0, 1, 2, 4, 8, or 16 days of similar daily feedings. Four hours after the final saline or fat infusion, plasma and jejunal mucosa were harvested; plasma levels of apo A-IV, triglycerides, and leptin were measured, as well as mucosal apo A-IV mRNA levels and biosynthesis of apo A-IV protein. In response to fat, plasma apo A-IV showed an initial 40% increase compared with saline-injected control rats; with continued daily fat feeding, the plasma A-IV response showed rapid and progressive diminution such that by 4 days, plasma A-IV was not different between fat- and saline-fed groups. Jejunal mucosal apo A-IV synthesis and mRNA levels also showed time-dependent refractoriness to fat feeding. However, the kinetics of this effect were considerably slower than in the case of plasma, requiring 16 days for completion. There was no correlation between plasma leptin or triglyceride levels and intestinal apo A-IV synthesis or plasma apo A-IV. These results indicate rapid, fat-induced, posttranslational adaptation of plasma apo A-IV levels and a slower, but similarly complete pretranslational adaptation of intestinal apo A-IV production, which are independent of plasma levels of leptin.

intestinal lipid absorption; triglycerides; leptin; gene expression; protein synthesis

APOLIPOPROTEIN A-IV (apo A-IV) is an intestinal synthesized, fat-responsive protein that has been implicated strongly in the control of food intake (12, 29) and in protection against atherosclerosis (3, 10, 11). Accordingly, there is growing interest in understanding the mechanisms underlying production and secretion of this protein. At present, these mechanisms are incompletely understood.

Although acute induction of apo A-IV by fat is well documented and remains the subject of ongoing investigation (17–21, 26), the effects of chronic fat feeding (an especially critical issue insofar as the role of apo A-IV in control of ingestive behavior and obesity are concerned) are less clear. Available evidence is inconclusive. In one study in humans, Weinberg et al. (30) reported that subjects ingesting a high-fat diet for 1 wk had elevated plasma apo A-IV levels; however, after 2 wk of high-fat feeding, plasma A-IV had returned to control (i.e., non-high-fat) levels. These authors proposed that autoregulation of plasma A-IV levels was likely at the level of catabolism; however, neither expression or synthesis of apo A-IV nor plasma A-IV metabolism were examined in their study. In an earlier study, Apfelbaum et al. (2) fed rats diets containing either butter fat or corn oil (30%, wt/wt) for 6 wk and reported apparent increases in jejunal mucosal synthesis of apo A-IV when compared with rats fed standard (i.e., low-fat) diet for 3 wk. Plasma levels of apo A-IV were not measured in this latter study. It is clear that further examination of effects of prolonged fat feeding on apo A-IV is necessary.

Recent studies indicate that adaptation of rats to high-fat diets shows reduced sensitivity to the satiating effects of both intestinal lipid infusion (6) and exogenous administration of some gut peptides, such as cholecystokinin (CCK) and bombesin (5). They also showed decreased ability of intestinal lipid and exogenous CCK to inhibit gastric emptying (7). The mechanisms for these effects are not certain but may involve an effect of chronic lipid on vagal responsiveness to lipid and/or CCK (4). Inasmuch as apo A-IV also has been proposed as a fat-elicited satiety signal (12, 29), it would be important to examine whether the aforementioned adaptive responses to chronic high-fat feeding extend to apo A-IV.

In the present experiments, we examined the response of plasma apo A-IV levels and jejunal mucosal synthesis and mRNA levels of apo A-IV, 4 h after a gastric lipid bolus, after graded durations of similar daily fat feeding. We sought answers to the following questions: 1) is there a change in the response of plasma apo A-IV to lipid under conditions of chronic fat ingestion; 2) if so, can it be explained by changes in intestinal production; and 3) in view of recent findings that exogenous leptin downregulates apo A-IV expression in the intestine (9, 24), is modulation of apo A-IV

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production and plasma levels associated with changes in plasma leptin levels?

MATERIALS AND METHODS

Male, Sprague-Dawley rats (250–275 g) with ad libitum access to standard diet and water were divided into groups and given daily, bolus intragastric injections of 3.6 ml of either saline or intralipid 20% fat emulsion [0.72 g, 814 μmol triglycerides (TG) equivalent] for 0, 1, 2, 4, 8, or 16 days. Assuming rats consume about 20 g of standard diet (4% fat, wt/wt) per day, this amount of daily supplement constituted about a 90% increase in daily fat intake. Before the final day of each time group, animals were deprived of food for 24 h after their gavage. On the final day, the fasted rats were given a final gavage of either lipid or saline. Four hours after this final gastric load, rats were anesthetized with halothane and jejunal mucosa and plasma were harvested as will be described.

Collection of plasma. Abdominal aortic blood was collected into heparinized tubes and 6.7 μg/ml leupeptin, 6.7 μg/ml pepstatin-A, 6.7 μg/ml chymostatin, 3 μg/ml aprotonin, and 6.7 μmol/ml phenylmethylsulfonyl fluoride (PMSF) were added to the plasma, which was then frozen at −80°C until analysis.

Analysis of plasma components. Plasma samples were assayed for apo A-IV using an ELISA method that has been validated against a previously published electroimmunoassay (16). Data herein are expressed as the %change from the respective saline-control values. Plasma leptin concentration was determined using an ELISA assay (Rat Leptin Correlate Kit, Assay Designs, Ann Arbor, MI), according to the manufacturer’s instructions. TG were measured using an enzymatic assay (Sigma 320-UV, Sigma Chemical, St. Louis, MO).

Measurement of jejunal mucosal apo A-IV mRNA. A 1-cm segment of proximal jejunum was placed on a glass plate over ice, and the mucosa was harvested by scraping with a glass slide. From this tissue, total RNA was isolated using the RNeasy RNA isolation kit (Qiagen, Valencia, CA) using the manufacturer’s suggested protocol. Isolated RNA was stored at −80°C until analysis.

Preparation and labeling of the apo A-IV cDNA probe and Northern blotting were described previously (18, 19); after autoradiography for apo A-IV signals, blots were stripped and reprobed for 18S ribosomal RNA (Ambion, Austin, TX). Relative levels of apo A-IV mRNA in control (saline) vs. fat-fed rats were determined by densitometric analysis, normalized to the 18S signal.

Measurement of jejunal mucosal apo A-IV protein synthesis. At the conclusion of the experimental period, rats were anesthetized with halothane, and synthesis of apo A-IV protein was measured as described previously (17–19). A 10-cm segment of proximal jejunum was isolated with ligatures. The loop was then incubated in situ for 10 min with luminal instilled [3H]leucine (0.5 μCi, Amersham/Pharmacia Biotech, Piscataway, NJ) and then removed and washed with ice-cold PBS containing 20 mM L-leucine. The washed segment was placed over ice and cut open longitudinally, and then the mucosa was harvested with a glass slide. The mucosa was homogenized in 1.5 ml PBS containing 1% Triton X-100, 2 mM leucine, 1 mM PMSF, 40 μg chymostatin, 160 μg leupeptin, and 6 μg pepstatin A. The homogenate was centrifuged at 100,000 g for 60 min in a Beckman 50.3 Ti rotor. An aliquot of the cytosolic supernatant was then subjected to precipitation using 10% trichloroacetic acid (TCA) and assayed for protein concentration using a modified Lowry procedure (Dc Assay; Bio-Rad, Hercules, CA). Total mucosal protein synthesis was expressed as the ratio of TCA-precipitable radioactivity to protein content. A separate aliquot of cytosolic supernatant was also subjected to specific immunoprecipitation of apo A-IV, using a polyclonal goat anti-rat apo A-IV antiserum. Preliminary experiments using reincubation of immunoprecipitated samples with additional antiserum established the amount of antiserum necessary to achieve quantitative immunoprecipitation. All subsequent immunoprecipitations were carried out using an excess of antiserum. Immunoprecipitated apo A-IV was resolved using SDS-PAGE followed by autoradiography. The radioactive band corresponding to apo A-IV was removed from the gel and solubilized using Solvable Tissue and Gel Solubilizer (Du Pont/NEN Research Products, Boston, MA), and the radioactivity was measured. Synthesis of apo A-IV was expressed as the amount of immunoprecipitable radioactivity as a percentage of total protein synthesis. This was a valid measure of apo A-IV synthesis, because total protein synthesis (ratio of TCA-precipitable radioactivity to cytosolic protein) was similar between treatments.

Statistical analysis. Effects of daily fat feeding on apo A-IV/18S mRNA levels and apo A-IV synthesis were analyzed by two-way analysis of variance. Plasma levels of apo A-IV and leptin were analyzed by one-way analysis of variance. Multiple comparison testing was performed using a multiple general linear model (31). The relationship between plasma leptin levels and jejunal mucosal apo A-IV synthesis was examined using linear regression analysis. Differences were considered significant if P < 0.05.

RESULTS

Plasma levels of apo A-IV. Four hours after an intragastric bolus of triglyceride, plasma levels of apo A-IV were elevated by 40% above those in rats given intragastric injections of saline (Fig. 1). This increase in plasma A-IV levels began to fall after only 1 day of fat feeding; by the fourth day of daily fat administration, plasma levels of apo A-IV in the fat-infused animals
were indistinguishable from those in saline-infused animals. By 8 days, and maintained for up to 16 days of daily fat feeding, apo A-IV plasma levels were completely refractory to a bolus fat load. In fact, from 8 to 16 days, A-IV levels in fat-infused rats were about 10–15% lower than in saline-infused rats, although this difference was not statistically significant.

**Jejunal mucosal apo A-IV mRNA levels.** Over the 16-day period, saline had no significant effect on mucosal gene expression of apo A-IV (Fig. 2). In rats receiving only a single bolus fat infusion, apo A-IV mRNA levels 4 h later were about twice that in saline-infused rats. This effect persisted, and in fact increased over 4 days of daily fat feeding, peaking at a fivefold difference between fat and saline groups at 4 days. By 8 days, the difference in A-IV response between the two groups had returned to about a twofold increase due to fat, but by 16 days, there was no difference in apo A-IV gene expression between the two groups.

**Jejunal mucosal synthesis of apo A-IV.** Saline administration had no effect on jejunal mucosal apo A-IV synthesis over the experimental period; at 4 h and 4 days, fat infusion produced significant increases in A-IV synthesis (3- and 2.6-fold, respectively) compared with controls (Fig. 3). By 8 days, the mean synthetic rate in fat-infused rats had fallen to a level that was not significantly different from the saline group, and this lower level of A-IV synthesis continued to 16 days.

**Plasma levels of leptin and TG.** There was a trend toward increasing plasma levels of leptin over the 16-day period, but this was not statistically significant (Fig. 4). There was no statistical difference between groups at any time point, although fat-injected rats showed a consistent trend toward lower circulating levels of leptin than saline-injected controls. To more fully evaluate the possible role of leptin in the control of apo A-IV synthesis, regression analysis was performed to determine the relationship between plasma leptin levels and apo A-IV synthesis (Table 1). No
significant relationship was found when both saline- and fat-treated rats were combined for analysis. Similarly, when the analysis was performed on the saline data alone, there was no significant relationship. In contrast, a significant, positive correlation was found between plasma leptin and jejunal apo A-IV synthesis in the fat-treated rats. This is opposite to what might have been expected if increasing leptin levels were responsible for the decrease in apo A-IV synthesis over the experimental period.

Four hours after fat feeding, plasma TG were about twofold higher than in response to saline, and this observation was consistent over the entire 16-day experimental period (Fig. 5). Thus the triglyceridemic effect of a fat meal was independent of the duration of prior lipid feeding.

**DISCUSSION**

The present studies confirm the observation in humans of Weinberg et al. (30) that the plasma levels of apo A-IV show an initial increase in response to high fat but then return toward basal levels with continued fat feeding. However, our results reveal that this adaptation is more complex than heretofore appreciated. Whereas fat-dependent modulation of intestinal apo A-IV gene expression is a long-term process, requiring weeks to be completed, surprisingly, plasma levels of apo A-IV become insensitive to continued lipid feeding much more rapidly, the plasma apo A-IV response 4 h after a single fat meal decreasing after only one previous exposure to lipid (i.e., 1 day rats), and becoming completely refractory to stimulation by lipid after 4 days of daily fat feeding. Time-dependent changes in jejunal synthesis of apo A-IV protein during chronic exposure to lipid were similar to those in mRNA levels, suggesting that 1) modulation of intestinal production of apo A-IV occurs at the pretranslational level, whereas 2) that of A-IV plasma levels occurs posttranslationally. Finally, these changes in intestinal production and plasma levels of apo A-IV were not correlated with levels of leptin in plasma, suggesting that changes in circulating leptin are unlikely to be responsible for chronic fat-induced adaptation of apo A-IV.

Several reports have linked apo A-IV with the control of food intake (12, 29). Although the precise role of A-IV in this regard is poorly understood, available evidence suggests that apo A-IV may be a fat-released satiety signal. Given the stimulatory effects of fat on apo A-IV production and secretion from the intestine (2, 16–19, 26), it is difficult to reconcile with the obesity-promoting effects of high-fat diets (14). However, most studies examining the effects of dietary lipid on production and release of apo A-IV have reported on the effects of acute lipid feeding only (2, 16–19, 26); comparatively few experiments have been conducted in which more chronic effects of fat on apo A-IV were tested.

Weinberg et al. (30) reported the first evidence for adaptation of plasma apo A-IV in response to prolonged fat feeding. They found that in human subjects on a high-fat diet for 1 wk, plasma apo A-IV was significantly elevated compared with baseline levels. However, after 2 wk on a high-fat diet, apo A-IV levels unexpectedly returned toward baseline. Intestinal production of apo A-IV could not be examined in their study, but on the basis of earlier experiments in the rat showing continued elevated synthesis of intestinal apo A-IV after 6 wk of high-fat feeding (2), Weinberg et al. concluded that the most likely explanation for the aforementioned changes in plasma apo A-IV was an increase in the metabolic clearance of this protein.

Apfelbaum et al. (2) placed rats on a 30% (wt/wt) fat diet for 6 wk and reported that compared with rats on a fat-free diet for 3 wk, jejunal apo A-IV synthesis was increased by 1.6–1.8-fold. In the same report, they showed that acute delivery of corn oil produced a greater effect on jejunal apo A-IV: 1.9–3.5-fold increases in A-IV synthesis compared with control. They also found that unlike acute experiments using bolus intragastric injections of corn oil, chronic high fat had no effect on ileal synthesis of apo A-IV. Intervening time periods were not examined, nor were plasma apo A-IV levels. Because these authors never intended to

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**Table 1. Regression analysis of relationship between plasma leptin levels and jejunal mucosal apo A-IV biosynthesis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + lipid</td>
<td>0.00025</td>
<td>0.247</td>
<td>0.197</td>
<td>1.210</td>
<td>0.280</td>
</tr>
<tr>
<td>Saline</td>
<td>-9.14 x 10^-5</td>
<td>0.262</td>
<td>-0.210</td>
<td>0.648</td>
<td>0.434</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.0007</td>
<td>0.203</td>
<td>0.522</td>
<td>5.251</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Data are results for 4 rats/group at each time point (4 h, 4, 8, 16 days). Regression performed on either full data set (i.e., saline- and fat-treated animals combined) or on the respective saline and lipid treatments. Apo A-IV, apolipoprotein A-IV.

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**Fig. 5. Plasma triglyceride levels 4 h after intragastric injection of either saline or fat after either 0, 4, 8, or 16 days of similar daily injections of either saline or fat (same rats as in Fig. 3). Data are means ± SE for 4 rats in each of saline- and fat-treated groups at each time point. *At a given time point, significant difference was noted between fat- and saline-treated groups (P < 0.001).**
specifically address the issue of adaptation, the conditions of their acute and chronic studies (e.g., lipid dose, physical form of test lipid, time since the rats' final lipid meal) were not similar. Thus unambiguous interpretation of their results as either supporting or not supporting adaptation is problematic.

Thus, at least in humans, there is strong evidence for adaptation of plasma apo A-IV in the face of elevated dietary fat intake. However, no previous study has specifically addressed whether such changes in plasma apo A-IV might be explained by changes in either production or metabolism of this protein. The intent of the present study was to examine whether intestinal production of apo A-IV would adapt to chronic ingestion of fat, and whether such regulation was correlated with events in plasma. Although A-IV is expressed in both intestine and liver in the rat (32), this study focused on intestinal production of apo A-IV. This was reasonable because it is well established that the intestine contributes the major proportion of circulating apo A-IV (32) and also in view of studies indicating that hepatic production of apo A-IV is unaffected by lipid feeding under conditions similar to those used here (unpublished observations). We chose to examine jejunal expression and synthesis of apo A-IV, inasmuch as expression of A-IV tends to be highest (2, 17) and most lipid transport occurs in the proximal gut (17). Our results indicate that while adaptation of intestinal apo A-IV gene expression and protein synthesis does indeed occur, it is too slow to account for the more rapid return of plasma apo A-IV to basal levels. Thus it appears that at least in the early stages of this adaptation (i.e., 1–4 days), the decrease in plasma apo A-IV cannot be explained by changes in intestinal production. It remains to be determined whether increased clearance of apo A-IV by the liver (8) or kidneys (27) or both is responsible for the rapid return of plasma apo A-IV to control levels.

Because of the kinetics of plasma apo A-IV, and in view of the traditional association of apo A-IV with TG-rich lipoproteins (i.e., chylomicrons) (15, 28), it was of interest to examine plasma levels of TG over the course of the experimental period. Similar to results reported in humans by Weinberg et al. (30), we found no evidence for adaptation of plasma TG. This strongly suggests that the rapid return of plasma apo A-IV levels to baseline is independent of (and therefore not secondary to) clearance of chylomicrons, making it likely that the changes in plasma apo A-IV we observed were mainly in the lipoprotein-free fraction of plasma. Although a change in high-density lipoprotein-associated apo A-IV cannot be ruled out in the present studies, the former conclusion seems more likely in view of previous findings that the lipoprotein-free fraction of plasma accounts for about 75% of circulating apo A-IV (13, 23). At present, the precise relative physiological roles of lipoprotein-associated vs. free apo A-IV remain unknown, although potentially important antiatherogenic effects recently have been ascribed to free apo A-IV (11).

Results from the experiments examining both mucosal mRNA levels and synthesis of apo A-IV protein indicate that over longer periods (8–16 days) of daily fat feeding, a second mechanism keyed to production of this protein may contribute to adaptation of plasma apo A-IV. Because the kinetics of mucosal apo A-IV synthesis followed those of mRNA levels, it seems clear that this is a pretranslational mechanism. It is not yet known whether it depends on changes in apo A-IV transcription rate or mRNA stability.

Recent studies (9, 24) have shown that exogenous administration of leptin to mice and rats decreases fat-stimulated gut expression and plasma levels of apo A-IV. Therefore, we examined whether adaptation of apo A-IV synthesis was associated with changes in circulating leptin. We observed, first, no significant effect of either treatment or time on plasma leptin levels. Second, we did not observe the inverse correlation between circulating leptin and fat-induced stimulation of apo A-IV synthesis, which might be expected if leptin were responsible for modulation of apo A-IV. In fact, regression analysis demonstrated a slight positive correlation between plasma leptin levels and apo A-IV synthesis in fat-treated rats. In light of the recent studies showing an inhibitory effect of exogenous leptin on apo A-IV (9, 24), our inability to find an association between circulating leptin and A-IV adaptation may be related to comparative plasma leptin levels between those previous studies and the present one. It is unknown how the plasma levels of leptin measured in our experiment compare with those that may have been produced in the aforementioned experiments using exogenous administration of leptin. However, it is clear that under the conditions of our study, adaptation of apo A-IV synthesis to chronic fat feeding was not accompanied by (and therefore presumably did not require) elevated levels of circulating leptin. Our results do not necessarily rule out a role for endogenous leptin in apo A-IV adaptation; unequivocal testing of this question will only be possible when leptin antagonists become readily available. However, at present, it seems likely that some other mechanism(s) is/are involved in the fat-induced adaptation reported here.

The mechanism responsible for the adaptation of apo A-IV's response to lipid during chronic fat ingestion is not known, but it is possible that apo A-IV may become less sensitive to lipid as a result of prolonged fat feeding. Although apo A-IV is exquisitely sensitive to low levels of fat in naive rats (17, 26), chronic exposure to higher lipid intakes may alter this sensitivity. Recent studies by Covasa and associates (4–7) provide a precedent for this idea. They have shown that both the food intake and gastric inhibitory effects of intestinal oleate and exogenous administration of CCK are reduced in rats previously adapted to high-fat diets (5–7). Moreover, they have shown that prolonged fat feeding reduces vagal sensory responses to both lipid and CCK and have proposed the general hypothesis that vagal responsiveness is altered during chronic fat feeding (4). This hypothesis may be of particular relevance to the control of apo A-IV, because at least part
of the A-IV response to lipid appears to be vagally mediated (18, 20, 21).

**Perspectives**

Our findings of adaptation of apo A-IV during chronic ingestion of fat have implications for two important issues relating to the effects of high-fat diets. Prolonged ingestion of high-fat diets is associated with overconsumption and obesity (14), but the underlying mechanisms are unknown. One possibility is that fat-induced satiety mechanisms might be altered in response to chronic fat ingestion such that their sensitivity or efficacy is diminished. One example is CCK, whose effects on food intake are diminished in rats adapted to high-fat diets (5). Another example is leptin, whose circulating levels in rats were recently shown to decrease in response to prolonged (i.e., 4 wk) feeding of a high-fat diet (1). Because apo A-IV has been found to be another potentially important fat-induced satiety signal (12, 29), downward adaptation of its production and circulating levels may contribute to the obesity-promoting effects of prolonged ingestion of lipid.

A second implication of the present findings relates to apo A-IV’s recently discovered antiatherogenic effects. Several in vivo studies in mice have clearly demonstrated protective effects of high-circulating levels of apo A-IV against the development of atherosclerotic lesions in models of both genetic and diet-induced atherosclerosis (3, 10, 11). If apo A-IV’s production and subsequent levels in plasma become refractory to lipid during chronic fat ingestion, the resulting relative deficit in circulating levels of this potentially important protective protein may contribute to the atherogenic effects of high-fat diets.

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