The prevalence of obesity is increasing worldwide, and rates have accelerated over the past decade. This fact indicates that the primary cause of obesity lies in environmental and behavioral changes, although genetic factors contribute to the propensity of an individual to become obese. One well-established important environmental factor predisposing to obesity is the amount of fat in the diet. Epidemiological studies have identified a significant positive correlation between average dietary fat intake and the incidence of obesity (8, 9). Importantly, recent data suggest that obese animals have blunted satiety, raising the possibility that defective signaling of satiety from gastrointestinal tract and/or central nervous system sources may contribute to the etiology of obesity (18).

Apolipoprotein A-IV (apo A-IV) is a protein secreted by the small intestine and liver in rats (1, 5, 20). Numerous studies have demonstrated that apo A-IV synthesis by small intestinal epithelial cells is stimulated by active lipid absorption (5, 13). Hayashi et al. (6) reported that the stimulation of apo A-IV production by lipid administration is associated with the formation of chylomicrons, because such stimulation could be abolished by Pluronic L-81, a potent inhibitor of the formation of chylomicrons. Recently, we have demonstrated (10) that apo A-IV is expressed in rat hypothalamus. Like the regulation of apo A-IV in the intestine, hypothalamic apo A-IV gene expression is also depressed by fasting and restored by lipid refeeding (10).

Although acute induction of apo A-IV synthesis in the intestine and hypothalamus by acute fat administration is well documented, the effects of chronic maintenance on a high-fat (HF) diet are less clear. In 1990, Weinberg et al. (17) first reported the adaptation of plasma apo A-IV in response to prolonged fat feeding in humans. They demonstrated that, after subjects consumed an HF diet for 1 wk, human plasma apo A-IV was significantly elevated compared with baseline levels, but that it returned toward baseline after the 2nd wk on the HF diet. Those authors proposed an autoregulation of plasma apo A-IV at the level of catabolism (17). In their study, however, neither apo A-IV synthesis nor plasma metabolism was examined.

Kalogeris and Painter (7) infused a fat emulsion intragastrically to overnight-fasted rats for 0, 1, 2, 4, 8, or 16 days. They observed an initial 40% increase in plasma apo A-IV in response to intragastrically administered fat compared with saline-infused controls. However, with continued daily fat feeding, the plasma A-IV response declined progressively such that, by 4 days, plasma A-IV levels were not different between fast- and saline-infused groups. Jejunal mRNA levels and mucosal apo A-IV synthesis had parallel time-dependent refractoriness to fat administration.

Apo A-IV has been proposed as a dietary fat-elicited satiety signal (2–4). Thus we hypothesize that, in response to increased fat ingestion, hypothalamic apo A-IV production increases and participates in the compensatory response to caloric excess. If this adaptive rise in apo A-IV were to become blunted in the setting of obesity, we further hypothesize that the response to caloric excess or fat ingestion may be blunted further, potentiating obesity. In the current study, we address the feasibility of this hypothesis in an established model of...
HF-induced obesity (19). The results indicate that HF-induced overeating and weight gain are associated with a change in the gene expression of apo A-IV in the hypothalamus.

METHODS

Animals. Male Long-Evans rats weighing 220–250 g (Harlan, Indianapolis, IN) were housed individually in plastic tub cages and maintained in a temperature-controlled (22 ± 1°C), humidity-controlled room on a 12:12-h light-dark cycle. They received pelleted rat chow (Teklad Sterilizable Mouse/Rat Diet, Harlan) and tap water ad libitum for 1 wk to allow for recovery from shipment and stabilization of body weight before being divided into dietary groups on the basis of comparable mean body weight. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati and were conducted in American Association for Accreditation of Laboratory Animal Care-approved facilities.

Diets. Two pelleted semi-purified, nutritionally complete experimental diets (AIN-93M) prepared by Dyets (Bethlehem, PA) were used. The HF diet contained 20 g fat/100 g diet by weight (19 g of butter oil and 1 g of soybean oil to provide essential fatty acids) and provided 19.34 kJ/g of diet that included 7.74 kJ/g as fat. The low-fat (LF) diet contained 3 g of butter oil and 1 g of soybean oil/100 g diet by weight and provided 16.12 kJ/g of diet, including 1.29 kJ/g as fat. The HF and LF diets differed only by fat and carbohydrate content, as described before (19), because protein and all of the essential minerals and vitamins were equalized to the amount required for rats per kilojoule (15).

Experimental groups. HF rats received ad libitum access to the HF diet, and LF rats received ad libitum access to the LF diet. Because HF and LF rats consumed different amounts of energy each day, two specific control groups were used. One control group, the pair-fed HF group (PHF), was given the HF diet each day, but in an amount limited to the average daily caloric consumption of the rats fed the LF diet ad libitum. That is, these rats received the same proportion of dietary fat as the HF rats but had their energy intake yoked to that of the LF rats. Mean daily energy intake of LF rats was calculated every 3 days, and that precise amount of energy, provided as the HF diet, was administered to the yoked rats each day. Over the course of 10 wk, the mean energy intake of the LF and the PHF rats was 83% of the energy intake of the HF rats.

Although PHF rats consumed the same amount of energy each day as LF rats, their food intake was limited, and these animals should be considered to be chronically food restricted. Because food restriction per se, independent of dietary conditions, could influence important variables of interest, we included a second control group, which consumed the LF diet and which was energy restricted by the same proportion (i.e., −17%) each day as the PHF rats (i.e., the PLF group). We also included an additional group of rats that consumed a nonpurified chow (CHOW) diet (Teklad Sterilizable Mouse/Rat Diet, Harlan) ad libitum to provide a link to the numerous reports that used CHOW diets as the only control for an HF diet. The difference between CHOW and semipurified diets includes nutrient content and energy density. Therefore, five groups (HF, LF, PHF, PLF, and CHOW) were included.

Table 1. Primers used in RT-PCR reaction

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
<th>Fragment Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-IV</td>
<td>5'-CTTTCGCAACAGAGCTAAAGG-3'</td>
<td>5'-TCTCCATTTCAGGCTGCTCG-3'</td>
<td>343 bp</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Liu et al. 2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-TGGCTCTCTCAGGCTGAGG-3'</td>
<td>5'-TGGCTGAGG-3'</td>
<td>386 bp</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Tatemoto 1982</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RT-PCR for apo A-IV mRNA. Total RNA from hypothalamus and intestine was isolated with Tri Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s suggested protocol. Total RNA concentration was determined spectrophotometrically at 260 nm. To ensure uniform and reproducible reaction conditions, both RT and PCR reaction reagents were prepared as master mixes and aliquoted into individual tubes before using them for each batch of reactions. Hypothalamic apo A-IV mRNA level was measured by competitive RT-PCR (10). RT was carried out with 200 ng of hypothalamic total RNA digested by DNase together with varying concentrations of apo A-IV competitor RNA, generated as previously described (10) in a volume of 50 μl with random hexamer primer and Moloney murine leukemia virus reverse transcriptase, according to the manufacturer’s guidelines (Amersham Pharmacia Biotech, Piscataway, NJ). The cDNAs were then amplified with 29 cycles. One round of amplification consisted of 30 s at 92°C, 45 s at 60°C, and 45 s at 72°C, where the final extension step lasted 7 min. Intestinal apo A-IV mRNA was determined by relative RT-PCR. The process for the relative RT-PCR was the same as described above, except that a 10-ng sample of total RNA but no apo A-IV competitor RNA was used in the RT reaction. The sequences of the primers for apo A-IV mRNA are depicted in Table 1. To determine whether equal amounts of total RNA had been added to the RT-PCR reaction, the housekeeping gene for glyceraldehyde-3-phosphate dehydrogenase (GAPDH, primers in Table 1) was used as an internal control.

PCR products were separated on 1.5% agarose gel containing Gelstar staining (FMC, Rockland, ME). The intensities of the bands were quantified using a PhosphorImager (Molecular Dynamics). For competitive RT-PCR, the intensity of the bands and the log of the ratio of amplified target PCR products (hypothalamic apo A-IV) to standard PCR products (apo A-IV competitor) were graphed as a function of the known amount of standard RNA added to the reaction (10). For relative RT-PCR, the level of amplified apo A-IV mRNA of intestine...
Data were analyzed by parametric statistics (repeated-measures ANOVA with time as the repeated measure, followed by paired *t*-test). Differences were considered significant when the probability of the difference occurring by chance was <5 in 100 (*P* < 0.05).

RESULTS

Body weight and plasma lipids. Body weight of the five groups of rats differed after 2 wk on the different diets, and after 10 wk the HF rats were significantly heavier, as depicted in Fig. 1, than all other groups (Fig. 1). As expected, PHF rats weighed less than HF rats (*P* < 0.05) and were not different from the LF rats (*P* = 1.005). PLF rats weighed less than LF rats, but the difference was not significant (*P* = 0.546). These results are comparable to what we have previously observed with these diets (19).

Plasma TG levels were higher in LF rats than in any of the other four groups over the entire course of the experiment (Table 2). The difference reached significance at week 8 compared with PLF (*P* = 0.019) and PHF (*P* = 0.029) rats and at week 10 compared with PLF rats (*P* = 0.032). TG levels in HF rat plasma were slightly lower than those in LF rats and slightly higher than those in CHOW, PLF, and PHF rats, but the difference among these five groups was not significant (Table 2).

Intestinal apo A-IV mRNA. We determined intestinal apo A-IV mRNA levels from rats in each of the five groups at any time period. There were no significant differences in gene expression of apo A-IV. Also, intestinal apo A-IV mRNA did not significantly correlate with body weight gain in any group of animals (data not shown).

Plasma apo A-IV. Plasma apo A-IV protein levels measured by ELISA were not affected by different diets over the course of the experiment. No significant correlations were found between plasma TG and intestinal or plasma apo A-IV mRNA levels, indicating that higher plasma TG levels in LF and HF rats were not associated with peripheral apo A-IV levels.

Hypothalamic apo A-IV mRNA. The effect of dietary fat on apo A-IV gene expression in the hypothalamus was dependent on the length of the dietary treatment. The HF rats had a slow and progressive diminution in hypothalamic apo A-IV gene expression, compared with the LF or CHOW animals, which attained significance after 10 wk (Fig. 2). After 10 wk on the diets, apo A-IV mRNA levels in the hypothalamus of the HF rats were reduced by 31% compared with the LF group (*P* < 0.05) and 43% compared with the CHOW animals (*P* < 0.01).

The response of hypothalamic apo A-IV gene expression to the stimulation of dietary lipid. As depicted in Fig. 3, intragastric infusion of lipid emulsion to fasted animals significantly increased hypothalamic apo A-IV mRNA content in all groups.
other than the HF rats \( (P < 0.05) \); i.e., hypothalamic apo A-IV in the HF rats is less responsive to dietary lipids than that in the other dietary groups.

**DISCUSSION**

As we have previously reported (19), rats with free access to the HF diet consumed more energy and became obese relative to rats with free access to a diet containing the same constituents but a lower proportion of fat. The purpose of these experiments was to test the hypothesis that, in response to increased fat ingestion, hypothalamic and intestinal apo A-IV production increases and participates in the compensatory response to caloric excess. However, this hypothesis has not before been tested directly. What we found was that hypothalamic apo A-IV gene expression decreased as a function of time on the HF diet and that the difference from controls was reliable by 10 wk. Interestingly, neither intestinal nor circulating apo A-IV levels were significantly altered, which is consistent with data in humans in whom, after 2-wk consumption of a high-fat diet, the plasma apo A-IV did not respond to lipids (8). Furthermore, Kalogeris and Painter (7) demonstrated that intragastric infusion of a fat emulsion to overnight-fasted rats increased intestinal expression and plasma apo A-IV levels only during the first few days. With continued daily fat infusion, these responses had a rapid and progressive diminution.

Apo A-IV is an apolipoprotein stimulated in response to the ingestion of lipids (6, 10). Several lines of evidence suggest that brain apo A-IV plays an important role in the regulation of feeding behavior. Intracerebroventricular administration of apo A-IV significantly inhibits food intake in a dose-dependent manner without eliciting signs of toxicity (2, 4, 11). Blocking the action of endogenous apo A-IV with a specific neutralizing antibody increases meal size, implying that endogenous apo A-IV exerts an inhibitory tone on feeding (3, 10). Centrally administered neuropeptide Y stimulates hypothalamic apo A-IV gene expression, suggesting that multiple factors interact to regulate apo A-IV levels in the hypothalamus (12). If brain apo A-IV is a physiological regulator of fat intake, the reduced hypothalamic apo A-IV levels in the HF animals observed in the present study would be predicted to reduce the satiety response and thereby contribute to their hyperphagia and/or fat preference.

An important question is whether the change in hypothalamic apo A-IV gene expression is a result of exposure to the HF diet or the resulting obesity. Our results showed that the rats consuming a restricted caloric intake of HF diet did not become obese and did not have decreased apo A-IV gene expression within the hypothalamus. We therefore conclude that the decrease in apo A-IV gene expression observed in the HF rats is secondary to increased caloric consumption and the consequent obesity, rather than to mere exposure to the HF diet itself. A very interesting question for future studies is whether one can increase the hypothalamic apo A-IV gene expression by diet restriction in animals that are already obese.

A change in hypothalamic apo A-IV gene expression could be caused by several factors. Given the sensitivity of hypothalamic apo A-IV gene expression to ingested lipids (10), the most obvious factor would be increased TG levels in the plasma of HF rats. This mechanism cannot be directly addressed in our study, because our animals were fasted for 20 h before measurement. An alternative cause of decreased hypothalamic apo A-IV gene expression was increased peripheral apo A-IV levels, which could negatively feed back to hypothalamic apo A-IV gene expression in the hypothalamus. However, this did not occur, because peripheral apo A-IV levels were not significantly altered after HF feeding. Future experiments will need to investigate the mechanism(s) by which increased caloric consumption and/or obesity decreases hypothalamic apo A-IV gene expression.

The alteration in apo A-IV mRNA in the hypothalamus is not the only factor that could contribute to the obesity caused by chronic HF feeding. Reduced apo A-IV signaling could also be the result of decreased response of hypothalamic apo A-IV to the dietary lipid. To test this hypothesis, we measured the hypothalamic apo A-IV mRNA levels after lipid infusion in all five groups of animals. We found that, unlike LF- or CHOW
fitted rats, the response of hypothalamic apo A-IV gene expression to a lipid infusion in 10-wk HF-fed rats was completely absent in HF rats. Although requiring further study, one implication of these data is that a manipulation to prevent hypothalamic apo A-IV level from diminution could be useful to treat increased weight that results from exposure to a diet that mimics the macronutrient content of the average American (8, 9).

It is worth noting that the response of animals to the HF diet is a subtle but cumulative effect, because it took over a 10-wk period for the HF rats to accumulate 50% more body fat than LF rats, while consuming just 17% more calories (19). The increase in obesity in our society is also the consequence of cumulative changes, which cause individuals to be in a mild positive energy balance year after year. Therefore, we may need to search for subtle effects, such as those observed here, to investigate the mechanisms by which specific diets increase body weight.

In conclusion, we have demonstrated that the chronic consumption of a HF diet significantly reduces apo A-IV gene expression and the response of apo A-IV to dietary lipids in the hypothalamus of rats. Since apo A-IV suppresses food intake, this response of hypothalamic apo A-IV could contribute to diet-induced obesity. Future studies will be needed to clarify the mechanisms by which increased body weight inhibits hypothalamic apo A-IV and to elucidate the specific hypothalamic areas where these changes occur.

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DISCLOSURES

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REFERENCES