

Fatty acid flux suppresses fatty acid synthesis in hamster intestine independently of SREBP-1 expression

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Abstract Hamsters were fed a control diet or diets containing palm, olive, safflower, or fish oil for 2 weeks. In villus cell populations from duodenum, jejunum, and ileum, rates of intestinal fatty acid and cholesterol synthesis were estimated, as were sterol regulatory element-binding protein (SREBP)-1a, SREBP-1c, SREBP-2, HMG-CoA synthase, fatty acid synthase, ATP citrate lyase, acetyl-CoA carboxylase mRNA levels, and SREBP-1 and SREBP-2 mass. Plasma cholesterol and triacylglycerol levels were increased in animals ingesting palm oil and decreased in animals ingesting fish oil. Fatty acid synthesis and fatty acid synthase activity were decreased in the proximal intestine of animals ingesting all the fat-containing diets. Intestinal cholesterol synthesis was unaltered. In animals fed fat, SREBP-1c gene expression was modestly increased in the duodenum of hamsters fed palm oil or olive oil, and decreased in animals ingesting safflower oil or fish oil. Fatty acid synthase, acetyl-CoA carboxylase, ATP citrate lyase, SREBP-2, and HMG-CoA synthase mRNA levels were not altered, nor were SREBP-1 or SREBP-2 mass. In the intestine, dietary polyunsaturated fatty acids suppress SREBP-1c mRNA without altering expression of its target genes, fatty acid synthase, acetyl-CoA carboxylase, or ATP citrate lyase. **Fatty acid influx decreases intestinal fatty acid synthesis by a posttranscriptional mechanism independent of the SREBP pathway.**—Field, F. J., E. Born, and S. N. Mathur. **Fatty acid flux suppresses fatty acid synthesis in hamster intestine independently of SREBP-1 expression.** *J. Lipid Res.* 2003. 44: 1199–1208.

Supplementary key words fat diet • cholesterol • triacylglycerol • gene expression

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate the transcription of several genes in both the cholesterol and fatty acid synthetic pathways [reviewed in ref. (1)]. SREBP-1a and SREBP-1c are derived from a single gene that uses an alternative transcriptional start site to produce the two isoforms of SREBP-1 (2). SREBP-2 is a product of a separate gene and has 50% homology to SREBP-1. All three SREBPs are synthesized as 125 kDa precursor proteins

bound to the endoplasmic reticulum. In times of cholesterol deficiency, a two-step proteolysis of the precursor protein releases a 68 kDa N-terminal end of the protein producing the active transcription factor. This “mature” or nuclear form enters the nucleus and binds to a 10 bp sterol regulatory element in promoter regions of target genes that result in enhanced transcription (3–7). Although there is overlap in their gene targets, SREBP-2 preferentially enhances genes in the cholesterol synthetic pathway, whereas SREBP-1, particularly 1c, tends to be more active in regulating genes of the fatty acid synthetic pathway (8–11).

Characterization and regulation of SREBPs have been extensively studied in several cell culture model systems and, in particular, liver of intact animals [reviewed in ref. (12)]. Pertinent to the objectives of the present study, previous studies have investigated the effect of dietary fat on the expression of SREBP-1 in liver (13, 14). The combined results of these studies demonstrate quite clearly that dietary polyunsaturated fats, either of the n-6 or n-3 class, decrease gene and protein expression of SREBP-1, which in turn are associated with a decrease in fatty acid synthase gene expression. These results have provided a potential mechanism for earlier observations that showed that dietary polyunsaturated fatty acids decrease transcription of genes responsible for controlling hepatic lipogenesis (15). In addition, from results in SREBP-1c transgenic animals, and in animals in which fasting, refeeding protocols were employed to induce fatty acid biosynthesis, it also seems clear in liver that SREBP-1c regulates fatty acid synthesis (8, 16). Thus, in liver, SREBP-1c regulates genes of fatty acid biosynthesis and, in turn, SREBP-1 expression is regulated by changes in dietary fat.

Although much is known about the regulation of cholesterol and fatty acid synthesis by dietary lipids in liver and how SREBPs might play a role in regulating these pathways, there remains a paucity of information regarding the regulation of these pathways in the intestine. This

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Abbreviation: SREBP, sterol regulatory element-binding protein.

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is unfortunate. The intestine is, after all, the first organ to encounter these dietary lipids (in very substantial amounts) and is responsible for transporting them in a form that can be taken up by peripheral tissues and liver. Our previous efforts have focused on understanding mechanisms for the regulation of intestinal cholesterol metabolism and what roles SREBPs play in this process (17, 18). There is, however, no information that addresses whether fatty acid synthesis is regulated by changes in fatty acid flux and what role, if any, SREBP-1 or SREBP-2 might have in this process in the gut. The present study was undertaken, therefore, to examine if changes in dietary fatty acid flux regulate intestinal fatty acid biosynthesis and whether SREBPs, particularly SREBP-1c, play a role in fatty acid synthesis in this organ.

MATERIALS AND METHODS

[³H]water (1 Ci/mmole) was purchased from ICN Biochemicals, Inc., Irvine, CA. Protease inhibitors and Tri Reagent were purchased from Sigma (St. Louis, MO). RNase-free DNase was purchased from Promega Corporation, Madison, WI. Superscript II RNase H-reverse transcriptase was obtained from Invitrogen Life Technologies, Carlsbad, CA. SYBR Green PCR mix was purchased from Applied Biosystems, Foster City, CA. All components for the diets except the oils were purchased from Harlan/Teklad Research Diets, Madison, WI. Corn oil, olive oil, and safflower oil were purchased from a local grocery store. Palm oil was obtained from Craftexpress, Memphis, TN. Fish oil (50:05 TG) was a generous gift from Ocean Nutrition Canada Ltd., Bedford, Nova Scotia, Canada.

Animals and diet

Male Golden Syrian hamsters weighing 90–120 g were purchased from Harlan Sprague-Dawley, Inc. They were maintained for a week on NIH-31 modified mouse/rat diet number 7013 (Harlan/Teklad) before starting the test diets. The animals were fed the diet ad libitum, and the light cycle was 0600–1800 h. Composition of the diets is shown in **Table 1**. The start of the dietary period was staggered in the hamsters so that all animals were fed the respective diets for exactly 14 days.

Fatty acid analysis of the diets and intestinal cells

The lipids from the diet or the cell fractions were extracted with 0.5 ml of hexane-2-propanol-water, 3:2:0.1 (v/v/v), followed by 4 ml of methanol. The solvent from the lipid extract of the diets was evaporated under nitrogen, and fatty acid methyl esters were prepared using 15% w/v boron trifluoride-methanol reagent. The cellular total lipid extract was applied on a silica gel plate and developed with hexane-diethyl ether-acetic acid-methanol (70:30:1:1,

v/v/v/v) to separate phospholipids from other lipids. The phospholipids from the silica gel were eluted by adding 4.5 ml water followed by 5 ml of methanol and 5 ml of chloroform. The mixture was mixed by vortexing, and phases were separated by centrifugation. The lower chloroform layer was collected, and the solvent was removed under nitrogen. The fatty acids in the phospholipid fraction were transesterified using 15% w/v boron trifluoride-methanol reagent. The fatty acid methyl esters were extracted in hexane and analyzed by gas liquid chromatography on a Supelco GP 10% SP-2330 on a 100:120 Chromosorb W AW column using a Hewlett-Packard 5890 gas chromatograph. The oven was programmed to an initial temperature of 180°C for 8 min followed by heating at 3°C/min to 210°C, where it was held for 15 min. The fatty acids were identified by comparison of retention time with samples of known fatty acid composition. **Table 2** shows the percent of the major fatty acids found in the respective diets.

Isolation of enterocytes

The cells along the villus axis were isolated from duodenum, jejunum, and ileum segments of the hamster intestine, as described previously by Field et al. (18).

Estimation of relative expression of mRNA levels by real-time RT-PCR

RNA was extracted from the intestinal cells using Tri reagent. Two micrograms of RNA were treated with RNase-free DNase to remove contaminating genomic DNA. After inactivating the DNase by addition of EGTA and heating at 65°C for 10 min, the RNA was transcribed to cDNA by SuperScript II RNase H-reverse transcriptase using random hexamers at 24°C for 10 min and 40°C for 4 h. After stopping the reaction by incubation at 90°C for 15 min, one twenty-fifth of the RT mixture was mixed with 2X SYBR green PCR master mix and gene-specific primers in a final volume of 20 µl. The primers were designed with Primer Express software. The primer pairs were selected to yield a single amplicon based on dissociation curves and analysis by acrylamide gel electrophoresis. **Table 3** provides the sequences of the primers used for the respective genes. Real-time quantitative PCR was performed in a model ABI Prism 7000 sequence detection system. The thermal cycler parameters were: hold for 2 min at 50°C and 10 min at 95°C for one cycle, followed by amplification of cDNA for 40 cycles with melting for 15 s at 95°C and annealing and extension for 1 min at 60°C. The values were normalized using 18S rRNA as an endogenous internal standard. A serial dilution of a standard was run on each plate for each mRNA and used to calculate the relative levels of the mRNA.

Immunoblot analysis

The methods for estimation of SREBP mass in the intestinal cells by immunoblotting have been described previously (18).

Cholesterol and fatty acid synthesis

Cholesterol and fatty acid synthesis were estimated by incorporation of tritiated water into these lipids 4 h after intraperitoneal injection of 0.25 ml of water containing 50 mCi of tritiated water.

TABLE 1. Composition of the diets

| | Diet | |
|---|---------|-------|
| | Control | Test |
| Palm oil, olive oil, safflower oil, or fish oil | | 94 g |
| Casein | 200 | 200 g |
| DL-methionine | 3 | 3 g |
| Corn oil | 20 | 20 g |
| Cellulose | 100 | 100 g |
| Corn starch | 612 | 400 g |
| Vitamin mix, AIN-93-VX (TD 94047) | 10 | 10 g |
| Mineral mix AIN-93G-MX (TD 94046) | 35 | 35 g |

TABLE 2. Fatty acid composition of the diets (% of total)

| Fatty Acid | Control | Palm Oil | Olive Oil | Safflower Oil | Fish Oil |
|------------|---------|----------|-----------|---------------|----------|
| C16:0 | 12.0 | 32.7 | 11.4 | 7.4 | 4.3 |
| C18:0 | 5.1 | 6.4 | 4.3 | 4.0 | 6.4 |
| C18:1n9 | 28.7 | 38.3 | 63.8 | 16.6 | 17.8 |
| C18:2n6 | 49.9 | 20.0 | 17.2 | 69.3 | 12.5 |
| C20:5n3 | 0.2 | | 0.1 | | 42.4 |
| C22:6n3 | | | | | 1.4 |

TABLE 3. Real-time RT-PCR primers for hamster mRNA

| | | bp | Reference or Accession number |
|------------------------|--|-----|-------------------------------|
| SREBP-1a | 5'-GGCTGTGGAACAGGCACTG-3' 5'-AGCTGGAGCATGTCTTCGATG-3' | 78 | (10) |
| SREBP-1c | 5'-GCGGACGCAGTCTGGG-3' 5'-ATGAGCTGGAGCATGTCTTCAAA-3' | 95 | (10) |
| SREBP-2 | 5'-AGCTGGCAAATCAGAAAAACAAG-3' 5'-GATTAAGTCTTCAATCTTCAAGTCCAC-3' | 93 | U12330 |
| HMG-CoA synthase | 5'-CCTATGACTGCATTGGGCG-3' 5'-CCCAGACTCCTCAAACAGCTG-3' | 101 | L00326 |
| Fatty acid synthase | 5'-AGCCCCCAAGTGCACAGTG-3' 5'-TGCCAATGTGTTTTCCCTGA-3' | 81 | AF356086 |
| Acetyl-CoA carboxylase | 5'-ACACTGGCTGGCTGGACAG-3' 5'-CACACAACCTCCCAACATGGTG-3' | 76 | AF356089 |
| ATP citrate lyase | 5'-TCCAAGAAGCCAAATCTTATCCTG-3' 5'-ATATTCATCAGCTTCTCC-3' | 107 | AF332051 |
| 18S | 5'-TAAGTCCCTGCCCTTTGTACACA-3' 5'-GATCCGAGGGCCTCACTAAAC-3' | 71 | Human-K03432 |

SREBP, sterol regulatory element-binding protein.

The radioactivity incorporated in the sterols and fatty acids was determined as described by Field et al. (18). The nmole of tritiated water incorporated in sterols or fatty acids/4 h/mg protein was calculated as follows:

$$\left[\frac{\text{(dpm in sterols or fatty acids)}}{\text{mg protein/fraction}} \right] / \left[\frac{\text{(dpm/nmole of water in plasma)}}{\text{mg protein/fraction}} \right] \quad (\text{Eq. 1})$$

Fatty acid synthase assay

Fatty acid synthase activity in the cytosol was assayed as described by Kumar and Dodds (19). Briefly, the intestinal cells were homogenized in 100 mM potassium phosphate buffer, pH 7, 0.1 mM sodium EDTA, and 1 mM DTT. The homogenate was centrifuged at 100,000 *g* for 30 min to obtain cytosol. The reaction mixture contained 100 μ g or 200 μ g cytosolic protein, 100 mM potassium phosphate buffer, pH 7, 0.1 mM sodium EDTA, 1 mM DTT, 0.3 mM NADPH, 0.05 mM acetyl CoA, and 0.15 mM malonyl CoA. The oxidation of NADPH at 340 nm was measured spectrophotometrically in a linear range at 1 min intervals.

Other analyses

An aliquot of the cell homogenate or total membrane preparation was taken and proteins were precipitated by adding 50 μ l of 0.15% deoxycholate and 0.45 ml of 10% trichloroacetic acid to remove substances that interfere in protein estimation. Protein content was then determined using the BCA method (Pierce Endogen, Rockford, IL). Triacylglycerol and cholesterol mass in plasma were estimated using the GPO Trinder reagent and INFINITY cholesterol reagent from Sigma. Cholesterol mass in the cellular lipids was determined as described earlier (18).

Statistical analysis

To determine whether significant differences existed among dietary fatty acids, the data were analyzed at $P < 0.05$ by one-way ANOVA and by the Student-Newman-Keuls method using SigmaStat software (SPSS, Inc., Chicago, IL).

RESULTS

Lipid parameters

Hamsters were fed ad lib for 2 weeks diets consisting of high carbohydrate and low fat (control), or diets in which the carbohydrate was replaced with the following oils

(10% wt/wt): palm oil (containing 33% of its fatty acids as 16:0, v/v), olive oil (64% 18:1, v/v), safflower oil (69% 18:2, v/v), or cholesterol-free fish oil (44% 20:5 and 22:6). **Table 4** shows the characteristics of these animals after the 2 week dietary period. Animals fed palm, olive, and safflower oils gained similar amounts of weight over the period. Like previous observations (13), despite having similar daily energy intakes, weight gain of animals fed the fish oil diet was similar to those animals fed the high-carbohydrate low-fat control diet.

Plasma cholesterol and triacylglycerol levels were analyzed. Plasma cholesterol and triacylglycerol concentrations were higher in the palm oil group than in controls (214 vs. 162 mg/dl and 262 vs. 168 mg/dl, respectively), and were lower in the fish oil group than in all other groups.

To examine if the type of dietary fat altered the fatty acid composition of intestinal membranes, the fatty acid composition of phospholipids isolated from cells prepared from duodenum, jejunum, and ileum of animals fed the respective diets was analyzed. For brevity, only data from the duodenum are shown, as the changes observed in the proximal intestine were reflective of the changes in

TABLE 4. Effect of diets on weight and plasma lipid levels

| | Weight Gain | Cholesterol/100 ml Plasma | Triacylglycerol/100 ml Plasma |
|---------------|---------------------------------|-------------------------------|-------------------------------|
| | <i>g</i> | | <i>mg</i> |
| Control | 6.7 \pm 0.93 | 162 \pm 6 | 168 \pm 22 |
| Palm oil | 9.9 \pm 0.70 ^a | 214 \pm 11 ^a | 262 \pm 43 ^a |
| Olive oil | 9.8 \pm 0.93 ^a | 155 \pm 8 ^b | 133 \pm 10 ^b |
| Safflower oil | 10.6 \pm 0.95 ^a | 150 \pm 11 ^b | 179 \pm 23 ^b |
| Fish oil | 6.5 \pm 0.91 ^{b,c,d} | 61 \pm 6 ^{a,b,c,d} | 79 \pm 10 ^{a,b,d} |

Hamsters were weighed at the beginning and end of the 14 days on the respective diets. Blood was collected just before sacrifice and cholesterol and triacylglycerol concentrations determined as described in Materials and Methods. The values represent mean \pm SE of 11–14 animals in each dietary group.

^a Values are significantly different from control at $P < 0.05$.

^b Values are significantly different from palm oil at $P < 0.05$.

^c Values are significantly different from olive oil at $P < 0.05$.

^d Values are significantly different from safflower oil at $P < 0.05$.

TABLE 5. Effect of diets on cellular phospholipid fatty acid composition

| | Control | Palm Oil | Olive Oil | Safflower Oil | Fish Oil |
|-----------------|------------|-------------------|-------------------|-------------------|-------------------|
| Duodenum | | | | | |
| C16:0 | 17.5 ± 0.5 | 20.4 ± 0.4 | 16.8 ± 0.3 | 16.6 ± 0.4 | 13.5 ± 0.7 |
| C18:0 | 18.7 ± 0.5 | 18.5 ± 0.5 | 19.5 ± 0.3 | 22.6 ± 0.2 | 25.5 ± 0.5 |
| C18:1n9 | 16.4 ± 0.5 | 18.9 ± 0.3 | 24.7 ± 0.6 | 8.2 ± 0.2 | 11.2 ± 0.3 |
| C18:2n6 | 32.9 ± 0.6 | 28.3 ± 0.7 | 25.3 ± 0.6 | 40.0 ± 0.9 | 11.7 ± 0.4 |
| C18:3n6 | 1.6 ± 0.6 | 2.4 ± 1.1 | 2.1 ± 0.9 | 0.2 ± 0.1 | 0.5 ± 0.1 |
| C18:3n3 | 0.3 ± 0.1 | 0.5 ± 0.2 | 0.9 ± 0.3 | 2.7 ± 1.0 | 2.9 ± 0.7 |
| C20:4n6 | 3.7 ± 0.3 | 3.6 ± 0.2 | 3.5 ± 0.1 | 2.7 ± 0.3 | 8.6 ± 0.9 |
| C20:5n3 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.0 | 13.6 ± 0.7 |
| C22:6n3 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.4 ± 0.2 | 0.8 ± 0.0 | 3.9 ± 0.3 |
| Saturated | 36.6 ± 0.4 | 39.3 ± 0.3 | 36.7 ± 0.3 | 39.5 ± 0.4 | 40.6 ± 1.1 |
| Monoenoic | 18.8 ± 0.9 | 19.5 ± 0.3 | 25.5 ± 0.6 | 9.8 ± 0.3 | 13.3 ± 0.5 |
| n-6 | 38.3 ± 0.8 | 34.4 ± 0.8 | 31.3 ± 0.5 | 43.2 ± 1.0 | 21.6 ± 0.8 |
| n-3 | 1.6 ± 0.1 | 1.8 ± 0.2 | 2.4 ± 0.4 | 3.7 ± 1.0 | 20.4 ± 0.6 |

Fatty acid composition of cellular phospholipids was analyzed by gas liquid chromatography as described in Materials and Methods. Each value is the mean ± SE from three animals on each diet. Only the major fatty acids are shown as a percent of the total. The values in bold print represent the predominate fatty acid in the respective oil.

jejunum and ileum (Table 5). To simplify and highlight the differences, the major fatty acid found in the respective fat diet is depicted in bold. It is clear from these results that intestinal phospholipids were enriched in the particular fatty acid that is most abundant in the fat diet. Total cholesterol content of whole homogenates prepared

from duodenum, jejunum, and ileum were similar in animals from all five dietary groups (unpublished results).

Effect of dietary fat on intestinal fatty acid and cholesterol synthesis

Following the dietary period, animals were injected into the peritoneum with tritiated water. After 4 h, the intestines were removed and divided into three equal segments corresponding to duodenum, jejunum, and ileum. Isolated cells were prepared from the three segments representing upper (fractions 1 and 2), middle (fractions 3 and 4), and lower (fraction 5) villus cells (18). The incorporation of tritiated water into fatty acids (Fig. 1) and cholesterol (Fig. 2) was determined in these cell fractions as an estimate of the rates of synthesis for these lipids. The bottom panels of Figs. 1 and 2 show the incorporation of labeled water into fatty acids and cholesterol in the different cell fractions along the villus axis. The top panels of the dietary group, rates of fatty acid synthesis were higher in cells of the lower villus compared with rates observed in the upper villus. Overall rates of fatty acid synthesis were similar along the length of the small intestine. Duodenal fatty acid synthesis was diminished by all fat-containing diets, relative to controls. Changes in jejunal fatty acid synthesis exhibited a pattern similar to that in duodenum, except

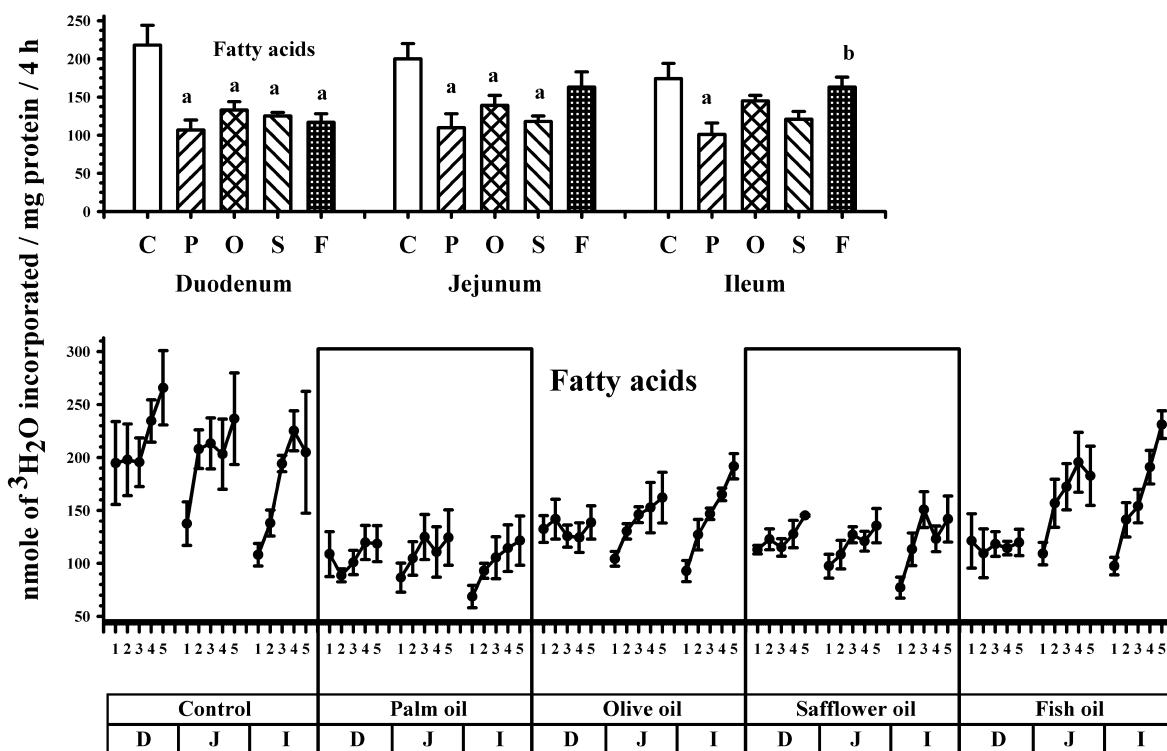


Fig. 1. Effect of dietary fat on intestinal fatty acid synthesis. Following the dietary period, tritiated water was injected into the peritoneum of animals. Four hours later the animals were killed and cell fractions from duodenum (D), jejunum (J), and ileum (I) were prepared as described in Materials and Methods. The incorporation of tritiated water into fatty acids was estimated as described in Materials and Methods. The lower panel represents fatty acid synthesis in cell populations along the villus axis with fractions 1 through 5 representing cells from villus tips to crypts. The upper panel represents the combined average of fatty acid synthesis in duodenum, jejunum, and ileum. Diets are represented by: C, control diet; P, palm oil; O, olive oil; S, safflower oil; and F, fish oil. The data represent the means ± SE of four animals on each diet. ^a Values are significantly different from control at *P* < 0.05. ^b Values are significantly different from palm oil at *P* < 0.05.

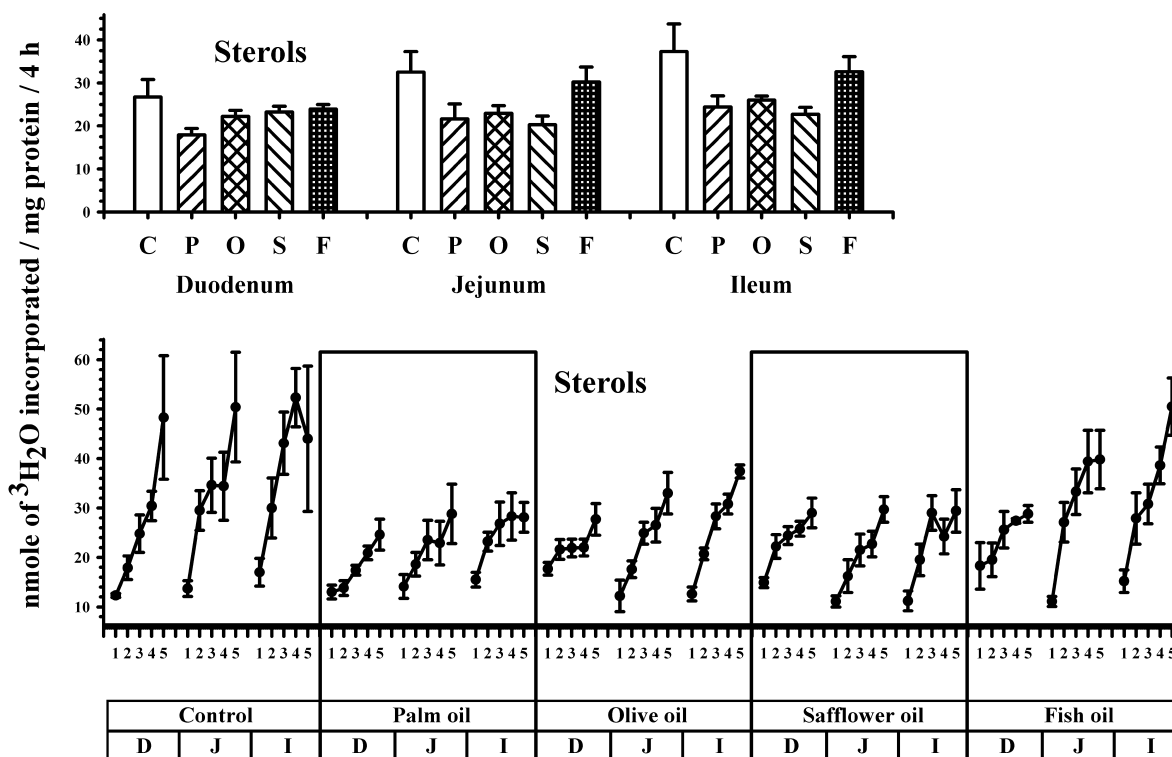


Fig. 2. Effect of dietary fat on intestinal cholesterol synthesis. The experimental design was the same as described for Fig. 1, except that the incorporation of tritiated water into cholesterol was determined.

that the fish oil group did not differ from controls. For ileal fatty acid synthesis, only the palm oil group was lower than controls. There were no significant differences in rates of intestinal fatty acid synthesis among the animals fed diets enriched in fat. Thus, increased fatty acid flux through the proximal intestine decreases the rate of fatty acid synthesis. Suppression of fatty acid synthesis by fatty acid flux is independent of the type of fat ingested.

Rates of cholesterol synthesis were also higher in cells of the lower villus, irrespective of the diet ingested (Fig. 2). The ingestion of fat did not significantly alter rates of intestinal cholesterol synthesis along the villus axis (bottom panel) or down the length of the gut (top panel).

Effect of dietary fat on intestinal SREBP gene expression and their target genes

To address the effects of fat ingestion on gene expression of SREBP-1a, SREBP-1c, and SREBP-2 and their target genes, fatty acid synthase, acetyl-CoA carboxylase, ATP citrate lyase, and HMG-CoA synthase, RNA was isolated from the intestinal cell fractions of duodenum, jejunum, and ileum of animals fed the respective diets. mRNA levels were estimated by quantitative RT-PCR. **Figure 3A** shows the mRNA levels in the cell populations along the villus axis, and **Fig. 3B** shows combined data for the three intestinal segments. In response to the diets, parallel changes were observed in mRNA levels for SREBP-1a and SREBP-1c, with significant changes occurring only within the duodenum. This is perhaps best illustrated in the combined data (Fig. 3B). In the duodenum,

mRNA levels of SREBP-1a and -1c were modestly increased in palm or olive oil animals compared with controls, whereas the ingestion of safflower oil had no effect. Expression of SREBP-1a and -1c was decreased in fish oil animals compared with animals ingesting all other oils. mRNA levels of SREBP-1c were decreased in duodenum of animals ingesting the polyunsaturated fats, safflower oil, or fish oil compared with palm or olive oil animals. The changes that were observed in gene expression of SREBP-1c and -1a occurred in all cell populations along the villus axis (Fig. 3A). For example, compared with mRNA levels of SREBP-1c in the duodenum of hamsters ingesting palm oil, mRNA levels were decreased in the upper, mid, and lower villus cells of hamsters ingesting fish oil.

In contrast, gene expressions for SREBP-2, HMG-CoA synthase, fatty acid synthase, acetyl-CoA carboxylase, and ATP citrate lyase were not altered by any of the fat-enriched diets (Fig. 3B).

Effects of dietary fat on SREBP-1 and SREBP-2 mass

To address whether fatty acid flux altered SREBP mass or the proteolytic processing of SREBP following the dietary period, total membranes were prepared from cells isolated from the three intestinal segments, and the amount of SREBP-1 and -2 mass was estimated (**Fig. 4** shows these data). Despite some variability, especially in the duodenum, neither the amounts of the precursor nor the mature forms of SREBP-1 or SREBP-2 were consistently altered by any of the diets.

Effect of dietary fat on fatty acid synthase activity

The results indicate that in the intestine, fatty acid influx suppresses fatty acid synthesis without decreasing gene expression of three key regulatory enzymes in the

fatty acid synthetic pathway, fatty acid synthase, acetyl-CoA carboxylase, and ATP citrate lyase. It could be argued that in animals ingesting fat, water incorporation into fatty acids could be decreased secondary to a lack of available

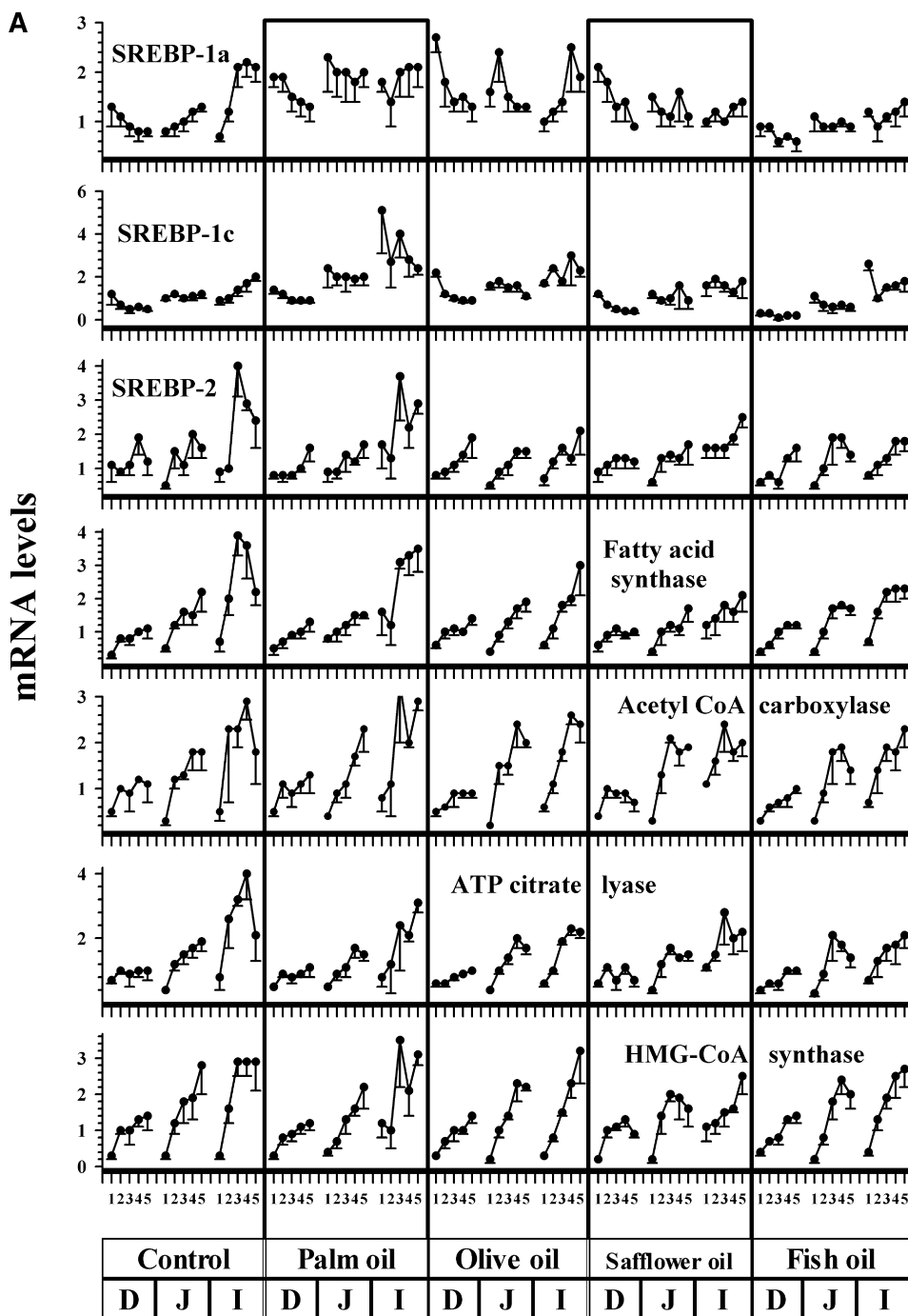


Fig. 3. Effect of dietary fat on intestinal mRNA levels of sterol regulatory element-binding protein (SREBP)-1a, SREBP-1c, SREBP-2, fatty acid synthase, acetyl CoA carboxylase, ATP citrate lyase, and HMG-CoA synthase. Following the dietary period, total RNA was extracted from individual cell fractions from duodenum, jejunum, and ileum. mRNA levels were estimated by quantitative RT-PCR as described in Materials and Methods. A: Shows mRNA levels in different cell populations along the villus axis where fractions 1 through 5 represent cells from villus tips to crypts. B: Depicts the combined average mRNA levels of the three intestinal segments. For duodenum, jejunum, and ileum, the values represent a relative change from control with control given a value of one. The data represent the means \pm SE of four animals on each diet. ^a Values are significantly different from control at $P < 0.05$. ^b Values are significantly different from palm oil at $P < 0.05$. ^c Values are significantly different from olive oil at $P < 0.05$. ^d Values are significantly different from safflower oil at $P < 0.05$.

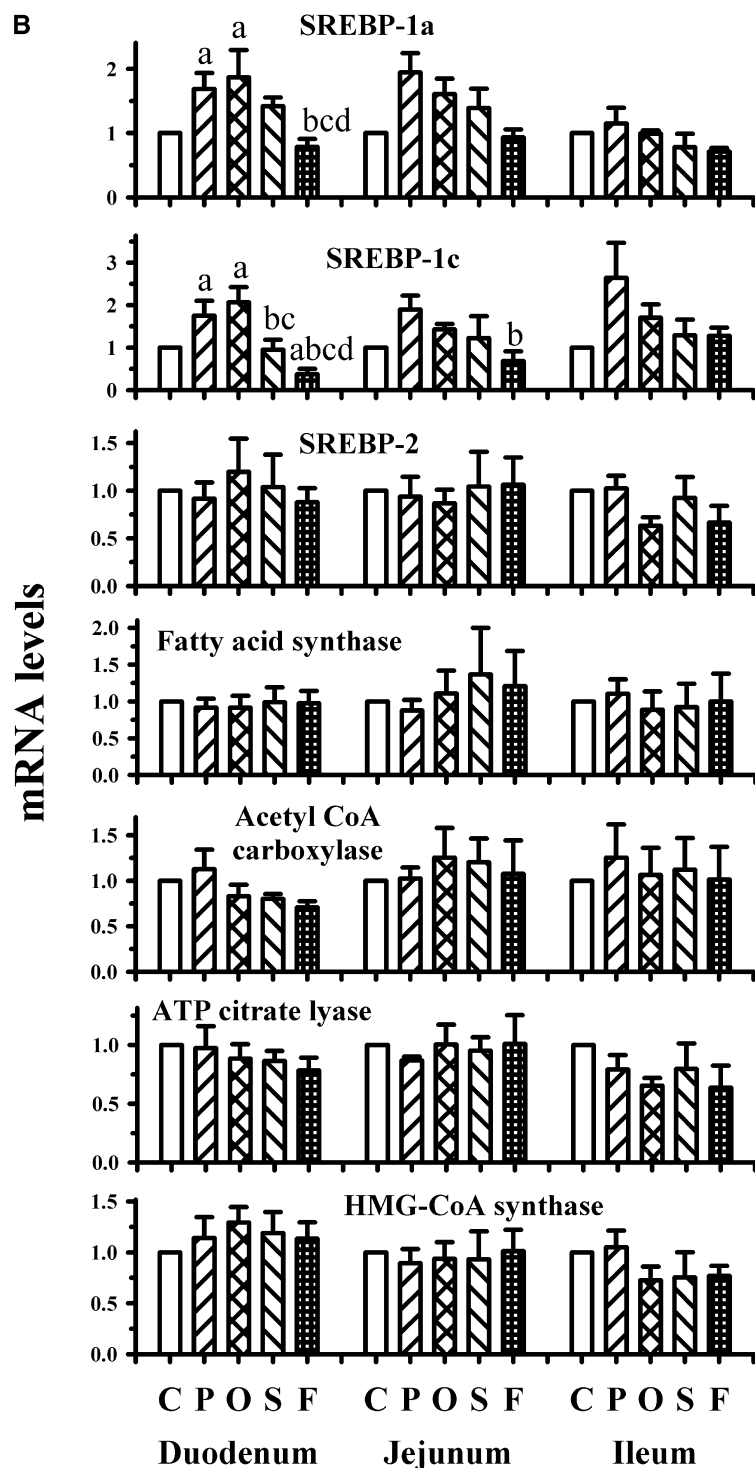


Fig. 3. *Continued.*

substrates for fatty acid synthase and may not be due to a decrease in fatty acid synthase activity per se. To address this, fatty acid synthase activity was measured in cytosol prepared from isolated cells obtained from duodenum and jejunum of animals on the respective diets (**Fig. 5** shows these data). Fatty acid synthase activity was significantly decreased in duodenum of animals ingesting fat compared with controls. Similar results were appreciated

in the jejunum, though the changes were more modest than those observed in the duodenum. Because the *in vitro* assay for fatty acid synthase contains saturating amounts of substrates, the results suggest that in the intact animal, the decrease in intestinal fatty acid synthesis by dietary fatty acids is not due to the limited availability of substrates for fatty acid synthase but is likely related to a direct effect upon the enzyme.

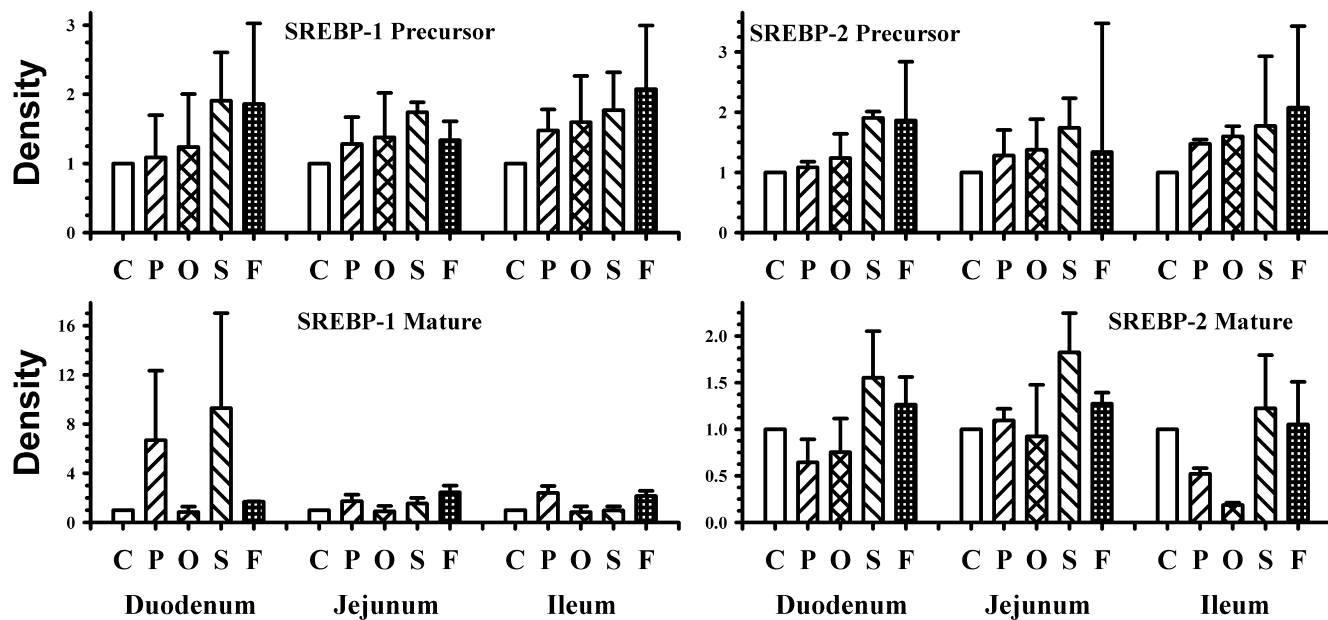


Fig. 4. Effect of diets on SREBP mass. Following the dietary period, total membrane fractions were prepared from cells isolated from the villus cell populations, and SREBP mass was estimated as described in Materials and Methods. The values represent a combined average of SREBP mass from cell populations of duodenum, jejunum, and ileum. The values are a relative change from control, with control given a value of one. The data represent the means \pm SE of three animals on each diet.

DISCUSSION

The results of the present study clearly demonstrate that the ingestion of fat decreases fatty acid synthesis in the proximal small intestine. To our knowledge, this has not been demonstrated previously. Was this finding unexpected? In liver, end-product feedback inhibition of cholesterol, fatty acid, and bile acid synthesis has been repeatedly shown (15, 20, 21). Should the intestine, another organ that receives a significant influx of lipids, differ from liver? Unlike hepatocytes, intestinal cells are not in the business of storing lipids for later use. Instead, the task of an intestinal cell is to transport lipids efficiently and rapidly from the exterior compartment to the interior compartment. Thus, it seemed plausible to speculate that transported lipids might bypass potential intracellular regulatory lipid pools and thus not cause end-product feedback inhibition. Indeed, an increased influx of cholesterol does not cause feedback inhibition of cholesterol synthesis in rodent intestine (18, 22). The fact that increased fatty acid flux suppressed intestinal fatty acid biosynthesis suggests that there is a mechanism by which fatty acids suppress their own synthesis in this organ. Unlike in the liver, however, the mechanism is not related to an alteration in SREBP mass or gene expression of rate-controlling enzymes in the fatty acid synthetic pathway (discussed below). In the proximal small intestine, all diets enriched in fat similarly suppressed fatty acid synthesis, suggesting that inhibition of fatty acid synthesis is independent of the type of fatty acid in excess. The data also suggest that suppression of fatty acid synthesis is likely related to the quantity of fatty acid flux. Only in the duodenum and jejunum, sites of most lipid transport, were rates of fatty acid synthesis decreased.

As mentioned, the results do not support a role for intestinal SREBP-1c or the SREBP pathway in regulating

fatty acid synthesis following fatty acid influx. In the proximal intestine, all dietary fats similarly suppressed fatty acid biosynthesis, and yet, only in animals ingesting fish oil was SREBP-1c gene expression decreased. In fact, SREBP-1c

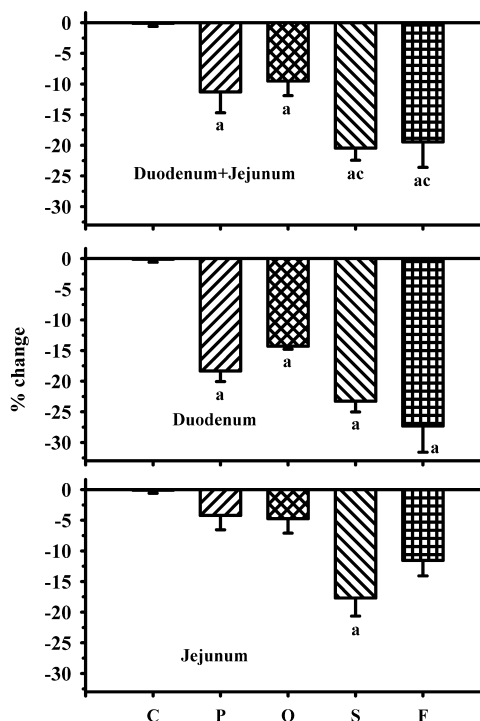


Fig. 5. Effect of diets on fatty acid synthase activity. Following the dietary period, cytosol was prepared from cells isolated from duodenum and jejunum. Fatty acid synthase activity was estimated as described in Materials and Methods. The values represent the means \pm SE from three animals on each diet, and are expressed as a change from control. ^a Values are significantly different from control at $P < 0.05$. ^c Values are significantly different from olive oil at $P < 0.05$.

gene expression was modestly increased in hamsters fed palm or olive oil. Moreover, SREBP-1 mass was not altered in the intestines of hamsters ingesting fat, including fish oil animals in which SREBP-1c gene expression was decreased. Furthermore, there was no evidence for proteolytic regulation of SREBP-1 by the diets, as the ratio of mature to precursor protein was not significantly altered. These observations, together with the fact that the target genes for SREBP-1, fatty acid synthase, acetyl-CoA carboxylase, and ATP citrate lyase were not altered by fat ingestion, support the notion that SREBP-1c is not an important factor in regulating fatty acid synthesis following fatty acid influx in the intestine. In an earlier study, we also observed a marked dissociation (in fact, reciprocal association) between intestinal SREBP-1c expression and fatty acid synthesis in hamster intestine following changes in intestinal cholesterol flux (18). Thus, in the hamster intestine, under conditions of increased fatty acid (and cholesterol) flux, there is no correlation between changes in rates of fatty acid synthesis and SREBP-1c expression. Similarly, in CaCo-2 cells incubated with micelles containing fatty acids 18:0 or 18:1, rates of fatty acid synthesis were decreased without altering SREBP-1 mass or mRNA levels of SREBP-1c, fatty acid synthase, or acetyl-CoA carboxylase (23). Yet, in that same study, polyunsaturated fatty acids 18:2, 20:4, 20:5, or 22:6 suppressed fatty acid synthesis and caused a parallel decrease in expression of SREBP-1c. In addition, the liver X receptor agonist, T0901317, enhanced SREBP-1c gene and protein expression, and resulted in a significant increase in fatty acid synthesis (23). Thus, results obtained in cell culture may not reflect what happens in the intact animal. This seems particularly true for the SREBP pathway, as fairly marked differences have been observed in results from cell culture models versus intact animals (1, 10). In the hamster intestine, the results are clear. Under conditions of changes in cholesterol or fatty acid flux, the SREBP pathway, and in particular SREBP-1c, has little, if any, role in regulating fatty acid synthesis.

In the liver, polyunsaturated fatty acids inhibit the expression of SREBP-1c compared with dietary saturated or monounsaturated fatty acids (13, 14). Moreover, n-3 polyunsaturated fatty acids appear to be more potent than n-6 polyunsaturated fatty acids in suppressing SREBP-1 expression. Our data in the intestine support the notion that polyunsaturated fatty acids, particularly n-3 polyunsaturated fatty acids, suppress SREBP-1c mRNA levels. SREBP-1c expression was significantly less in the proximal intestine of safflower oil and fish oil animals compared with palm oil and olive oil animals, with fish oil being more potent. Similarly, in CaCo-2 cells, micelles containing the polyunsaturated fatty acids 18:2, 20:4, 20:5, or 22:6 cause a decrease in mass and gene expression of SREBP-1 (23). Thus, although our data do not support a role of intestinal SREBP-1c in controlling fatty acid synthesis following lipid flux, our results do suggest that polyunsaturated fatty acids, particularly n-3 polyunsaturated fatty acids, suppress mRNA levels of SREBP-1c. In cell culture, it is thought that polyunsaturated fatty acids interfere with liver X re-

ceptor ligand activation of SREBP-1c (24). Whether this mechanism for regulating SREBP-1c expression occurs in vivo is unknown.

The ingestion of fat did not alter total cholesterol mass, rates of cholesterol synthesis, or gene expression of SREBP-2 or HMG-CoA synthase. One might have expected an increased demand for cholesterol to ensure normal assembly and secretion of triacylglycerol-rich lipoproteins. Assuming that the amounts of biliary and dietary cholesterol remain similar, an obvious possible source is newly synthesized sterol. Because this did not occur, it suggests that biliary cholesterol and/or basal rates of intestinal cholesterol synthesis are sufficient to meet the cholesterol needs of the intestinal cells during times of enhanced lipoprotein production. Failure of increased fatty acid flux to enhance intestinal cholesterol synthesis was noted in earlier studies, also performed in hamsters (25, 26), and recently in CaCo-2 cells (23). Micellar fatty acids of different chain lengths and degrees of saturation did not alter SREBP-2 gene or protein expression, suggesting that the SREBP-2 pathway was not being activated following fatty acid influx. The results again are clear. In hamster intestine, increased dietary fatty acid flux does not change the expression of SREBP-2 nor does it alter cholesterol synthesis. **FIG**

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