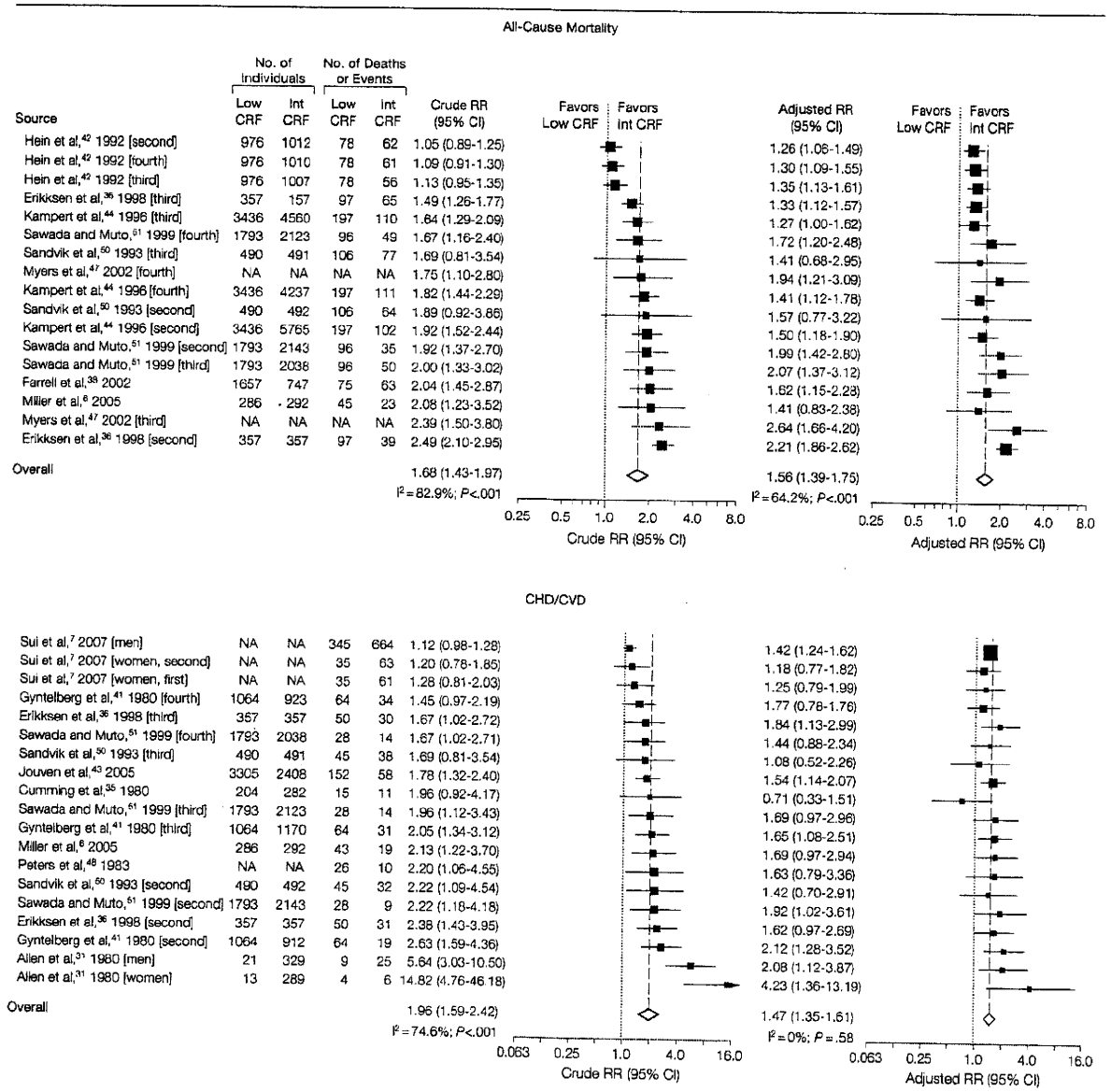
cant risk reduction of all-cause mortality.[60] However, using CRF may be preferable to using physical activity as risk predictors because 1 prior study[61] suggested that physical fitness was more strongly correlated with CHD than physical activity.

According to the results reported herein, the minimum CRF level that is associated with significantly lower event rates for men and women is approximately 9 and 7 METs (at 40 years old), 8 and 6 METs (at 50 years), and 7 and 5 METs (at 60 years), respectively. This means that women and men younger than 60 years

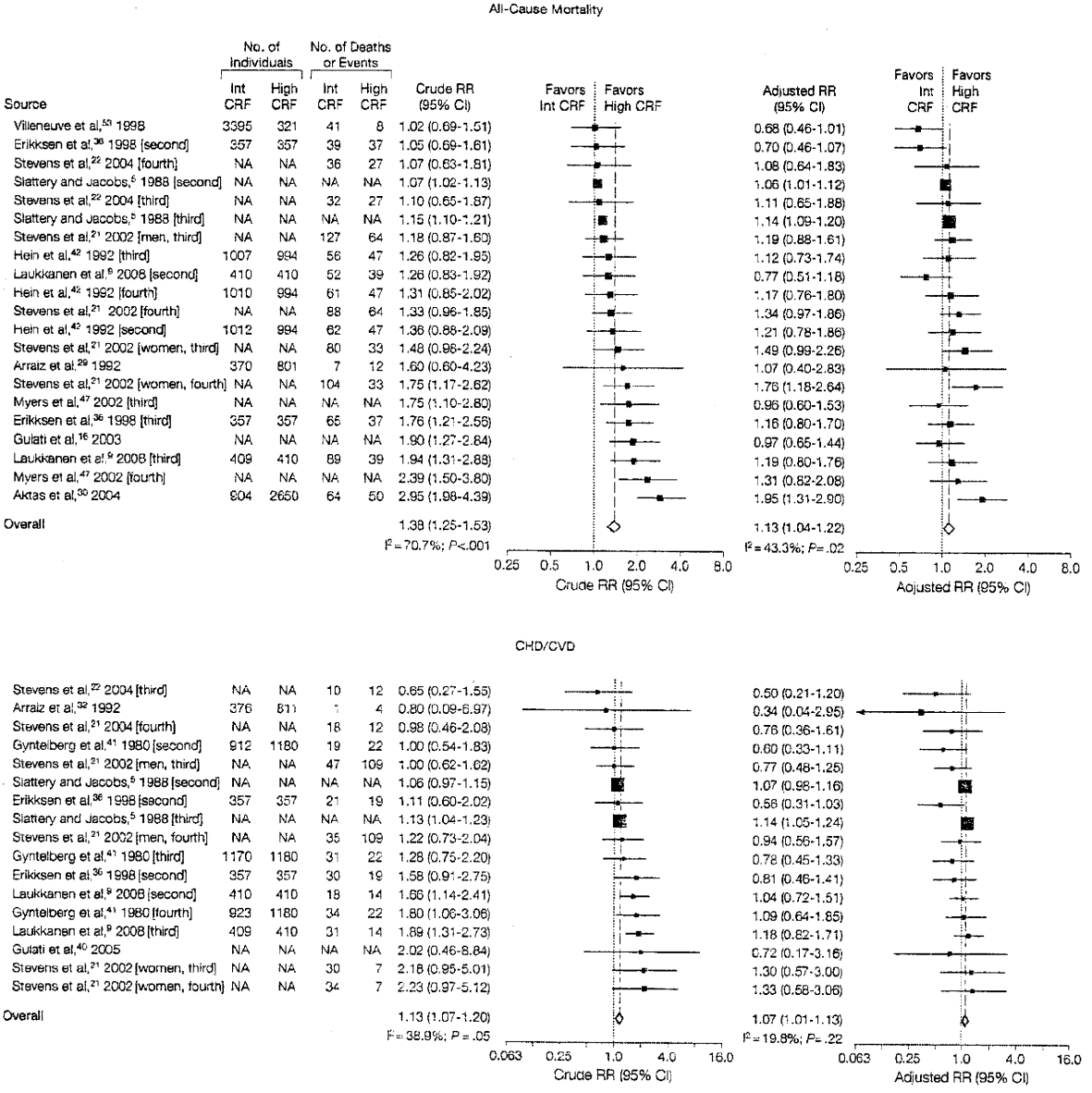
Figure 4. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Low vs Intermediate CRF



CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; Int, intermediate; MET, metabolic equivalent; NA, not available; RR, risk ratio. Low and intermediate CRF categories were defined as less than 7.9 METs and 7.9 to 10.8 METs of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively. The words first, second, third, and fourth in brackets represent comparisons between the lowest CRF category and the highest, second, third, or fourth CRF category in the relevant study.

would need to complete stage I (1.7 mph at gradient 10°) and stage II (2.5 mph at gradient 12°), respectively, of the standard Bruce protocol, which is one of the most commonly used treadmill tests in clinical settings.[14] If the CRF level is expressed in terms of walking speed, men around 50 years of age must be capable of continuous walking at a speed of 4 mph and women must continuously walk at 3 mph for prevention of CHD,[17] with the assumption that the anaerobic threshold is 50%

Figure 5. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Intermediate vs High CRF



CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; Int, intermediate; MET, metabolic equivalent; NA, not available; RR, risk ratio. Intermediate and high CRF categories were defined as 7.9 to 10.8 METs and 10.9 METs or more of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively. The words second, third, and fourth in brackets represent comparisons between the second, third, or fourth highest CRF category and the highest CRF category in the relevant study.

to 60% of MAC.[62] It is possible that consideration of low CRF as a major coronary risk factor could be put into practical use in the clinical setting through identification of low exercise tolerance by exercise stress testing or in daily life by the speed at which a person can walk before experiencing exhaustion.

Some cross-sectional population studies have suggested that higher aerobic fitness is associated with more favorable coronary or cardiovascular risk factor profiles[63,64]; therefore, the association between CRF and the risk of all-cause mortality and CHD/CVD could potentially be explained by residual confounding by established risk factors. Our sensitivity analyses indicated that a weaker association was observed between a 1-MET higher level of MAC and risk reduction of CHD/CVD, but not all-cause mortality, in studies with adjustment for smoking or more comprehensive risk factors. This finding suggests that better CRF is independently associated with longevity, while the inverse association between CRF and risk of CHD/CVD is explained partly by established coronary risk factors.

Limitations of this meta-analysis must be considered. First, a possible misclassification bias might affect our results. Misclassification bias could occur in transforming the reported CRF data into MET units. However, all of the prediction equations used in our analyses for estimating MAC have already been validated and are commonly used. Another possible misclassification bias is due to the fact that the definitions of low, intermediate, and high CRF were fundamentally based on study-specific CRF classifications, which varied from study to study but were not based on a standard cutoff. Fortunately, we could assign every exposure in each study to 1 of the 3 categories, which did not overlap with few exceptions, although MAC values in each category are approximately 1 MET smaller than those based on a general standard (eg, data from the National Health and Nutrition Examination Survey[65]). Therefore, the possibility of misclassification bias due to those 2 reasons should be limited. Second, Begg or Egger tests suggested publication bias. However, trim and fill analyses to incorporate potentially existing negative studies did not change the general result, although the risk estimates were moderately attenuated. Nevertheless, this possibility was not fully excluded by this analysis.

Based on the findings of our meta-analysis, we suggest for future research (1) further development of a CHD prediction algorithm (eg, Framingham Scores[66]) that would consider both CRF and the classical coronary risk factors to allow physicians to use CRF as a major risk factor in clinical settings; (2) cost-effectiveness of exercise testing for assessing CRF from the viewpoint of primary prevention of all-cause mortality and CHD; and (3) a clinical trial to determine whether an intervention that improves CRF by exercise reduces the risk of all-cause mortality and CHD.

In conclusion, better CRF was associated with lower risk of all-cause mortality and CHD/CVD. A 1-MET higher level of MAC was associated with a 13% and 15% risk reduction of all-cause mortality and CHD/CVD, respectively. The minimal MAC value for substantial risk reduction in persons aged 50 (SD, 10) years was estimated to be 8 (SD, 1) METs for men and 6 (SD, 1) METs for women. We suggest that CRF, which can be readily assessed by an exercise stress test, could be useful for prediction of CHD/CVD and all-cause mortality risk in a primary care medical practice.

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Influence of Fat and Carbohydrate Proportions on the Metabolic Profile in Patients With Type 2 Diabetes: A Meta-Analysis

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OBJECTIVE — The effects of dietary macronutrient composition on metabolic profiles in patients with type 2 diabetes have been inconsistent. This meta-analysis aimed to elucidate the effect of replacing dietary fat with carbohydrate on glucose and lipid parameters in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — We searched for randomized trials that investigated the effects of two kinds of prescribed diets (a low-fat, high-carbohydrate [LFHC] diet and a high-fat, low-carbohydrate [HFLC] diet); in these studies, energy and protein intake did not differ significantly between the two dietary groups. Nineteen studies that included 306 patients met our inclusion criteria. Median diet composition of carbohydrate/fat in the LFHC and HFLC diets was 58%/24% and 40%/40%, respectively.

RESULTS — Changes in values for A1C, fasting plasma glucose (FPG), and total and LDL cholesterol did not differ significantly between the LFHC and HFLC groups. However, the LFHC diet significantly increased fasting insulin and triglycerides by 8% ($P = 0.02$) and 13% ($P < 0.001$), respectively, and lowered HDL cholesterol by 6% ($P < 0.001$) compared with the HFLC diet. There were positive associations among the magnitude of changes in FPG, fasting insulin, and triglycerides for the diets analyzed. However, stratified analysis indicated that the increase in triglycerides was insignificant when accompanied by energy intake restriction.

CONCLUSIONS — Our findings suggested that replacing fat with carbohydrate could deteriorate insulin resistance while the adverse effect on triglycerides from the LFHC diet could be avoided by restricting energy intake to a degree sufficient for the attainment of weight reduction.

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Medical nutrition therapy (MNT) is the most important aspect of diabetes treatment (1). Optimizing energy intake and macronutrient composition are especially major topics in MNT. Whereas it is well-known that caloric restriction is essential for the achievement of good glycemic and lipid profiles, mainly through weight loss, the optimal dietary macronutrient composition for patients with type 2 diabetes remains controversial.

Since a high-protein diet is not recommended for diabetic patients because of the risk of nephropathy (1), macronutrient composition is mainly regulated by the carbohydrate-to-fat (C/F) ratio. Conventionally, restricting fat intake has been promoted to decrease energy intake and reduce weight (2). However, a low-fat diet, inevitably accompanied by high carbohydrate intake, may increase postprandial plasma glucose, insulin, and triglyceride levels (1). Therefore, the benefit of raising the dietary C/F ratio on metabolic control in type 2 diabetes has not been established. The effects of a low-fat, high-carbohydrate (LFHC) diet or a high-fat, low-carbohydrate (HFLC) diet in which total energy and protein intake are consistent in patients with type 2 diabetes have often been compared. The aim of this meta-analysis is to systematically compare the effects of LFHC and HFLC diets on glucose and lipid control in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

We searched MEDLINE (between 1966 and 2007) and the Cochrane Library Central Registry of Controlled Trials (between 1984 and 2007) for relevant publications using the following medical subject heading terms: diabetes and (food or diet). We examined reference lists of those publications to identify additional studies suitable for our purpose. We restricted the search to randomized controlled trials published in English. We searched for studies of the effects of two kinds of prescribed diets differing according to proportions of carbohydrate and fat under conditions that the prescribed total energy and protein intake did not differ significantly between groups of patients with type 2 diabetes. Trials in patients with type 1 diabetes were excluded. We designated one diet as the LFHC diet, which was defined as having a relatively high C/F ratio, and the other as the HFLC diet, which had a relatively low C/F ratio. As shown in detail in Table 1, in examining these studies, we found that the C/F ratio ranged from 0.60 to 1.56 for the HFLC diets and from 1.67 to 7.30 for the LFHC diets.

Among the studies identified, we included only randomized controlled trials with measurements of fasting plasma glucose (FPG) and fasting insulin and intervention periods of ≥1 week. Both parallel-group and crossover designs

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Table 1—*Descriptive statistics of studies included in the meta-analysis*

	Intervention period (weeks)	Dropout (%)	LFHC		HFLC		Age (years)	Men (%)	BMI	Using antihyperglycemia agents (%)	Diabetes duration (years)
			n	C/F/P (%)	*n*	C/F/P (%)					
Campbell et al. (1994; ref. 13)	2	N/A	10	55/22/23	10	40/37/23	55	100	26.5	10	5
Chen et al. (1995; ref. 14)	6	N/A	9	55/30/15	9	40/45/15	49	67	27.5	N/A	N/A
Coulston et al. (1989; ref. 15)	6	0	8	60/20/20	8	40/40/20	66	63	25.5	75	N/A
Fuh et al. (1990; ref. 16)	2	N/A	11	60/20/20	11	40/40/20	58	100	25.8	100	N/A
Garg et al. (1992; ref. 17)	3	N/A	8	60/25/15	8	35/50/15	63	100	30	0	N/A
Garg et al. (1994; ref. 18)	6	0	42	55/30/15	42	40/45/15	58	79	28.1	100	N/A
Heilbronn et al. (1999a; ref. 19)	12	17	12	73/10/17	10	50/32/18	58	27	32.9	58	5
Heilbronn et al. (1999b; ref. 19)	12	15	12	73/10/17	13	50/32/18	58	20	33.1	52	6
Lovejoy et al. (2002a; ref. 20)	4	12	30	58/27/15	30	46/39/15	54	43	33	47	N/A
Lovejoy et al. (2002b; ref. 20)	4	12	30	58/27/15	30	46/39/15	54	43	33	47	N/A
Luscombe et al. (1999; ref. 21)	4	25	21	53/21/26	21	42/35/23	57	67	30.4	76	6
Miyashita et al. (2004; ref. 22)	4	N/A	11	63/10/27	11	40/35/25	52	73	27	0	N/A
Parillo et al. (1992; ref. 23)	2	0	10	60/20/20	10	40/40/20	53	70	26.7	50	8
Parillo et al. (1996a; ref. 24)	2	0	9	60/20/20	9	40/40/20	48	N/A	24.7	0	6
Parillo et al. (1996b; ref. 24)	2	0	9	60/20/20	9	40/40/20	50	N/A	24.6	100	8
Rodriguez-Villar et al. (2000; ref. 25)	6	25	12	55/30/15	12	45/40/15	N/A	N/A	27.9	N/A	6
Rodriguez-Villar et al. (2004; ref. 26)	6	15	22	55/30/15	22	45/40/15	61	54	28.3	N/A	N/A
Rusmussen et al. (1994; refs. 27, 28)	3	N/A	15	50/30/20	15	30/50/20	57	67	27	47	6
Sestoft et al. (1985; ref. 29)	1.4	N/A	8	50/30/20	8	42/36/22	48	50	22.7	0	5
Simpson et al. (1982; ref. 30)	4	N/A	10	60/22/18	10	35/47/18	58	N/A	26.2	80	6
Storm et al. (1997a; ref. 31)	3	0	15	50/30/20	15	40/45/15	53	53	29.7	73	6
Storm et al. (1997b; ref. 31)	3	0	15	50/30/20	15	40/45/15	53	53	29.7	73	6
Median	4	6	12	58/24/20	12	40/40/20	55	65	27.7	52	6
Minimum	1.4	0	8		8		48	20	22.7	0	5
Maximum	12	25	42		42		66	100	33.1	100	8

C/F/P, proportion of carbohydrate/fat/protein to total energy of the prescribed diet; N/A, not assessed.

were included. Studies that included an intervention with a change in the content or quality of carbohydrate such as an increase in fiber and whole grains were excluded because such diets are high in fiber, which in itself ameliorates glycemia and lipemia regardless of changes in the C/F ratio (3,4). Studies of very-low-calorie or enteral (not oral) diets and those in which the dosage of hypoglycemic agents was changed during the intervention period were also excluded. One of three reviewers extracted all studies that met the eligibility criteria, and a second reviewed all extracted data. When necessary, disagreement was resolved by discussion with a third author.

Extracted data included features of the study design (i.e., crossover or parallel design and presence of a washout period), intervention periods, characteristics of patients (mean age, BMI, percent men, and percent those using hypoglycemia agents). Other extracted data regarded the characteristics of each diet, such as macronutrient composition; a weight-loss diet, which was defined as caloric restriction resulting in weight reduction; a weight-maintenance diet, which was defined by a weight change of ≤1 kg during the intervention period, and a monounsaturated fat (MUFA) diet within the HFLC-diet group, which was defined as the addition of MUFA to the HFLC diet. We also extracted baseline and final means and statistical dispersions of each group for the following metabolic profiles: A1C, FPG, fasting insulin, total cholesterol, fasting triglycerides, LDL cholesterol, HDL cholesterol, and 2-h postprandial levels of glucose and insulin. If VLDL cholesterol but not triglyceride data were provided, the triglyceride value was calculated by multiplying VLDL cholesterol × 5 according to the Friedewald formula (5). Also, if HbA_1 but not A1C data were provided, A1C was estimated by the relation between HbA_1 and A1C concentrations according to the methodology of Kilpatrick et al. (6). If necessary, measures of means and dispersion were approximated from figures in the articles using an image scanner (CanoScan LiDE 500F [resolution 600 dpi]; Canon, Tokyo, Japan). Study quality was assessed according to the scale described by Jadad et al. (7), with each included trial evaluated according to randomization, double blinding, withdrawals, and dropouts.

The effect on each metabolic profile, which is expressed as the mean difference between LFHC- and HFLC-diet groups in individual studies, was calculated by subtracting the change from baseline to final values in the HFLC-diet group from that in the LFHC-diet group. The SE of the

change from baseline values was directly extracted from the reported data or estimated from the SEs of the baseline and final values in the LFHC- and HFLC-diet groups, assuming a correlation of 0.5 between the baseline and final measures within each group, according to the formula of Follmann et al. (8), as follows:

$$\sqrt{(SE_{baseline})^2 + (SE_{final})^2 - 2 \times 0.5 \times (SE_{baseline}) \times (SE_{final})}$$

We chose the percent change from baseline values because the mean baseline and final values in patients in each study were highly skewed. To estimate percent change, we divided each change from baseline values and its SE by the baseline value. When no baseline value was reported, as in some crossover studies, we summarized the intervention effect by the ratio of the difference in final values between LFHC- and HFLC-diet groups to the final value in the HFLC-diet group and assumed that the baseline SE was equal to the final SE. This method of estimating percent change has limitations, especially in studies without washout periods. Therefore, we performed a sensitivity analysis to examine the effect of these studies on the results.

All percent changes were firstly pooled with a fixed-effects model (9). For each outcome measure, influence analysis was conducted to detect an outlier (i.e., a single estimate with an extreme result), which influenced overall outcome. Study heterogeneity was statistically assessed by Q statistics (9). If heterogeneity was significant, the percent changes were secondarily re-pooled with a random-effects model (9). Publication bias was assessed using two formal methods: Begg's test (10) and Egger's test (11). The trim-and-fill technique (12) was used to investigate the impact of any suggested bias.

We also calculated the weighted mean difference (WMD) in individual trials by multiplying each percent change by the inverse of its SE squared. We ecologically examined the mutual association among each metabolic effect of the LFHC diet compared with the HFLC diet by Spearman's correlation analyses among WMDs.

To investigate the effect of study characteristics, stratified analyses were performed for the following possible confounders: study design (i.e., whether each trial used a crossover design and, if so, whether the trial had a washout period or data on baseline values), intervention period (<4 vs. ≥4 weeks), percent the study of female sex (<50 or ≥50%), mean age (<55 vs. ≥55 years), BMI (<28 vs. ≥28 kg/m^2), percentage using hypoglycemia agents (zero vs. above zero), C/F ratio in the LFHC (>3 vs. ≤3) and HFLC (>1 vs. ≤1) groups, prescription of the MUFA diet (yes vs. no), and prescription of a weight-loss or weight-maintenance diet. We additionally conducted linear multivariable regression analyses to determine whether the characteristics of the patients were independent predictors that influenced the effect of the LFHC diet versus that of the HFLC diet. In this analysis, age, BMI, and the carbohydrate proportion in the LFHC and HFLC diets were entered as continuous variables. A P value of ≤0.05 was considered statistically significant. All analyses were performed with STATA software version 10 (STATA Corporation, College Station, TX).

RESULTS

Descriptive statistics on studies included in the meta-analysis (Table 1)

Of 2,203 potentially relevant publications based on search terms and 22 references obtained from manual searches, 19 (13–31) met the inclusion criteria. Four articles (19,20,24,31) included two trials in one study, and two articles (27,28) used the same cohort. Finally, 22 trials (306 patients) were included in our analyses. Studies included in the current analysis had intervention periods ranging from 10 days to 6 weeks and patient numbers ranging from 8 to 42. Means ± between-study SDs for the mean study characteristics from 22 trials were as follows: age 55 ± 5 years, percent men 63 ± 23, BMI 28 ± 3 kg/m^2, percent using hypoglycemia agents 52 ± 31, and diabetes duration 6 ± 1 years.

Ten studies (15,18–21,23–26,31) described the number of dropouts, and nine (13,14,16,17,22,27–30) did not. The dropout rate ranged from 0 to 25%. None of the 19 articles described methods of randomization, which led to a low quality score for the trial. A crossover design was used in 17 studies (13–18,20,21,23–31) (with 19 trials), whereas a parallel design was used in two studies (19,22) with three trials. Median carbohydrate/fat proportion of total energy (C/F ratio) in the LFHC and HFLC diets was 58%/24% (2.4) and 40%/40% (1.0), respectively. Three studies (19,22,26) with 4 trials prescribed a weight-loss diet, and 11 studies (13,14,17–19,21,23–25,27,28) with 11 trials provided a MUFA diet to the HFLC-diet group.

Overall effects of the LFHC diet compared with those of the HFLC diet on metabolic outcomes and study heterogeneity

Table 2 provides a summary of pooled estimates of various outcome measures. There were no significant differences in the reduction in A1C, total cholesterol, and LDL cholesterol between the LFHC and HFLC diets. However, the LFHC diet produced significant increases in fasting insulin and triglycerides levels of 8.4% (P = 0.02) and 13.4% (P < 0.001), respectively, and a significant reduction in HDL cholesterol compared with that associated with the HFLC diet. Two-h glucose and insulin values were higher in the LFHC-diet group than in the HFLC-diet group by 10.3% (P < 0.001) and 12.8% (P < 0.001), respectively.

Influence analyses indicated that there were a few outliers for percent change in total (22), HDL (22), and LDL (29) cholesterol (see online appendix Tables A1 and A2, available at http://care.diabetesjournals.org/cgi/content/full/dc08-1716/DC1). When these trials were omitted from the analyses, percent change in total cholesterol, HDL cholesterol, and LDL cholesterol significantly changed from −0.0% (95% CI −2.1 to 2.0) to −1.6% (−4.5 to 1.3; P = 0.03), from −10.4% (−12.2 to −8.6) to −5.6% (−2.9 to −8.4; P < 0.001), and from −3.0% (−6.3 to 0.4) to −0.1% (−4.1 to 3.8; P = 0.001), respectively. These outlying trials comprised a large part of study heterogeneity in percent change in total, HDL, and LDL cholesterol (22.2, 59.1, and 53.0%, respectively.) Therefore, they were excluded from the following analyses for the outcome that they affected. After omission of these outliers, there was no evidence of significant study heterogeneity (P > 0.4 for all outcomes).

Relationships among the magnitude of effects on metabolic profiles

Ecological analyses showed trends indicating that the WMD in FPG was positively associated with that in fasting insulin (r = 0.45; P = 0.04) and triglycerides (r = 0.59; P = 0.004) and that the WMD in fasting insulin and triglycerides was mutually associated (r = 0.43; P = 0.04). These associations remained signif-

Table 2—*Overall percent changes resulting from LFHC versus HFLC diet on metabolic profiles and data on publication bias and their likely effect on the estimates*

	A1C	FPG	2-h glucose	Fasting insulin	2-h fasting insulin	Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol
Trials (*n*)	10	22	10	22	9	20	22	20	16
Overall percent change	−1.5	0.3	10.3	8.4	12.8	1.6	13.4	−5.6	0.1
95% CI	−5.3 to 2.3	−2.8 to 3.4	6.7–13.9	1.3–15.6	5.2–20.4	−1.3 to 4.5	7.1–19.8	−8.4 to −2.9	−3.8 to 4.1
P	0.70	0.87	<0.001	0.02	<0.001	0.27	<0.001	<0.001	0.94
Publication bias									
Begg's	0.80	0.82	0.25	0.30	0.40	0.85	0.48	0.75	0.86
Egger's	0.47	0.30	0.12	0.13	0.16	0.26	0.75	0.08	0.92
Trim and fill									
Fill*								7	
Adjusted†								−7.6	
95% CI								−10.2 to −5.0	

*Studies (*n*) added by the trim-and-fill method. †Percent change after adjustment for publication bias by the trim-and-fill method. Begg's, Begg's adjusted rank correlation test; Egger's, Egger's regression asymmetry test.

icant after adjustment for whether a weight-loss diet was prescribed (FPG vs. fasting insulin, $r = 0.58$ and $P = 0.004$; FPG vs. triglycerides, $r = 0.44$ and $P = 0.04$; and fasting insulin vs. triglycerides, $r = 0.44$ and $P = 0.04$).

Test of publication bias

Table 2 also shows data on publication bias and its likely effect on estimates of outcome according to the trim-and-fill method (12). There was a relatively strong suspicion of publication bias for HDL cholesterol (Egger's test, $P = 0.08$ for HDL cholesterol; recommended level of significance, $P \leq 0.10$ [32]). According to results of the compensatory trim-and-fill method, the effect of publication bias would slightly underestimate the adverse effect of the LFHC diet.

Sensitivity analysis

Results of our stratified analysis to detect characteristics of studies and patients included in our analyses that might have modulated study outcomes are shown in Table 3. Of the 17 studies with a crossover design, 9 with 10 trials (14–16,21,23–26,29) did not include a washout period, which could lead to an underestimation due to a carryover effect (33). Moreover, none of these studies had baseline data. However, the effect of these nine studies on results was not significant for any of the measures.

The elevation in fasting insulin was remarkable (17.1%; $P = 0.001$) in LFHC diets with a C/F ratio ≥3 (in this case, an LFHC diet with ≥60% carbohydrate and ≤20% fat of total energy) while the C/F ratio in the LFHC diet did not influence triglycerides. There was a greater elevation in triglycerides (21.0%; $P<0.001$) with the LFHC diet when the LFHC diet and MUFA diet were compared; i.e., MUFA was replaced with carbohydrate. However, the magnitude of the elevation in fasting insulin did not differ between the MUFA diet and non-MUFA diet (i.e., regardless of dietary fat quality). Whereas a larger elevation in triglycerides was observed in trials limited to weight-maintenance diets, the LFHC diet did not significantly elevate triglycerides compared with the HFLC diet when only trials with weight-loss diets were examined (i.e., diets for weight loss) ($P = 0.48$).

The elevation in fasting insulin was greater in younger and leaner patients in response to the LFHC diet compared with that in response to the HFLC diet. Moreover, mean age and BMI were independent predictors of percent change in fasting insulin. Multiple regression analysis indicated that every −1 kg/m^2 of BMI and −1 year of age were independently associated with a greater elevation in fasting insulin by 2.6% ($P = 0.002$) and 1.7% ($P = 0.005$), respectively. For patients not taking antihyperglycemic drugs, the LFHC diet could be more harmful for fasting insulin than the HFLC diet. However, because only a few studies included patients not receiving antihyperglycemic drugs, the results should perhaps be interpreted with caution.

CONCLUSIONS — Although central to MNT, the influences of various dietary C/F ratios on glycemic control and lipid profiles in patients with type 2 diabetes have not been systematically reviewed. Our meta-analysis is the first to quantify the effect of the LFHC diet compared with that of the HFLC diet on each metabolic outcome.

Our results fundamentally support current dietary guidelines (1) stating that replacing fat with carbohydrate significantly elevates postprandial glucose and insulin levels when total energy intake is consistent. We additionally found that the LFHC diet significantly elevated fasting insulin compared with the HFLC diet, with marked elevations noted when the C/F ratio was ≥3. Moreover, there were significantly positive relationships among the change in FPG and the magnitude of the elevation in fasting insulin and triglycerides, independent of energy restriction for weight control.

Postprandial hyperglycemia with postprandial hyperinsulinemia and failure to maintain glucose homeostasis are often clustered in insulin-resistant individuals, who are representative of those with type 2 diabetes (34). This suggests that an LFHC diet is unfavorable compared with an HFLC diet for insulin-resistant patients, at least when energy intake is consistent. However, our findings do not support the benefit of an extremely high-fat diet because the carbohydrate proportion in the HFLC diets included in our analyses was ≤50%. Moreover, we cannot comment on the possible benefit of a high-carbohydrate diet with a high-fiber component because we excluded studies investigating the effect of such a diet. Moreover, there is concern that increased fat intake ad libitum may promote weight gain (35). It is worth repeating that total caloric intake and nu-

Table 3—*Stratified analysis to examine the effects of characteristics of studies and patients on each metabolic profile*

	FPG		Fasting insulin		Triglycerides		Total cholesterol		HDL cholesterol		LDL cholesterol	
	N	Percent change (95% CI)	*N*	Percent change (95% CI)	*N*	Percent change (95% CI)	*N*	Percent change (95% CI)	*N*	Percent change (95% CI)	*N*	Percent change (95% CI)
Study with washout period or baseline data												
Neither*	10	0.9 (−4.6 to 6.3)	10	9.0 (−1.7 to 19.7)	10	18.2 (7.3–29.1)	9	0.7 (−4.2 to 5.5)	9	−6.8 (−10.3 to −3.2)	5	−0.7 (−8 to 6.6)
Others†	12	0.0 (−3.8 to 3.7)	12	8.0 (−1.6 to 17.6)		11.0 (3.2–18.8)	11	2.2 (−1.5 to 5.8)	11	−4.0 (−8.2 to 0.3)	11	0.5 (−4.2 to 5.1)
Period <4 weeks	10	1.3 (−2.9 to 5.5)	10	15.2 (3.1–27.3)	10	15.8 (4.9–26.6)	10	1.7 (−3 to 6.4)	10	−7.3 (−10.9 to −3.6)	6	0.8 (−7.5 to 9.1)
Period ≥4 weeks	12	−1 (−5.5 to 3.6)	12	4.9 (−3.9 to 13.7)	12	12.2 (4.4–20)	10	1.6 (−2.1 to 5.3)	10	−3.6 (−7.7 to 0.6)	10	0.0 (−4.5 to 4.4)
<50% of subjects female	13	0.4 (−3.3 to 4.1)	13	6.2 (−3.4 to 15.9)	13	13.7 (5.8–21.6)	11	2 (−1.8 to 5.9)	11	−3.4 (−8.1 to 1.2)	10	0.6 (−4.6 to 5.8)
≥50% of subjects female	5	1.6 (−4.6 to 7.8)	5	11.5 (−0.1 to 23.2)	5	15.1 (2.8–27.3)	5	1.8 (−3.8 to 7.4)	5	−7.4 (−11 to −3.7)	4	−0.5 (−7 to 6.1)
Mean age <55 years	10	−0.2 (−4.1 to 3.7)	10	17.2 (6.7–27.8)‡	10	12.7 (4.6–20.8)	8	1.2 (−3.9 to 6.2)	8	−5.8 (−9.2 to −2.4)	4	−0.6 (−7.5 to 6.3)
Mean age ≥55 years	11	1.2 (−3.9 to 6.4)	11	1.7 (−8.2 to 11.7)‡	11	15.1 (4.5–25.7)	11	1.9 (−1.7 to 5.5)	11	−5.6 (−10.3 to −0.8)	11	0.7 (−4.2 to 5.5)
BMI <28.0 kg/m²	12	1.9 (−2.8 to 6.6)	12	18.2 (7.6–28.8)‡	12	12.5 (4.6–20.4)	10	1.2 (−3.5 to 5.8)	10	−7.8 (−11.6 to −4.1)	6	−0.9 (−8.6 to 6.9)
BMI ≥28.0 kg/m²	10	−1 (−5.1 to 3.1)	10	0.3 (−9.4 to 9.9)‡	10	15.1 (4.6–25.7)	10	1.9 (−1.8 to 5.6)	10	−3.1 (−7.1 to 0.9)	10	0.5 (−4.1 to 5)
Taking hypoglycemic agents	18	−0.6 (−4.1 to 2.9)	18	4.4 (−3.8 to 12.7)§	18	15.4 (6.9–23.8)	17	1.5 (−1.5 to 4.6)	17	−3.1 (−6.6 to 0.4)	15	0.1 (−3.8 to 4.1)
Not taking hypoglycemic agents	4	2.9 (−3.3 to 9.1)	4	20.7 (6.3–35.1)§	4	10.9 (1.4–20.5)	3	2.6 (−6.7 to 11.8)	3	−9.4 (−13.7 to −5.1)	1	0 (−31.8 to 31.8)
C/F ratio in LFHC ≥3	8	0.5 (−5.5 to 6.5)	8	17.1 (5.7–28.6)§	8	9.3 (−0.9 to 19.4)	7	−0.1 (−5.4 to 5.1)	7	−4.6 (−10.9 to 1.6)	4	−3.1 (−11.4 to 5.2)
C/F ratio in LFHC <3	14	0.2 (−3.4 to 3.8)	14	2.9 (−6.2 to 12.1)§	14	16 (8–24.1)	13	2.4 (−1.1 to 5.9)	13	−5.9 (−8.9 to −2.8)	12	1.1 (−3.4 to 5.5)
C/F ratio in HFLC ≤1	12	0.2 (−3.8 to 4.2)	12	8.1 (−4 to 20.2)	12	18.7 (8.3–29.1)	11	1.2 (−2.7 to 5)	11	−4.2 (−9.1 to 0.6)	8	−0.6 (−6.4 to 5.2)
C/F ratio in HFLC >1	10	0.4 (−4.5 to 5.2)	10	8.6 (−0.2 to 17.5)	10	10.4 (2.4–18.3)	9	2.2 (−2.2 to 6.6)	9	−6.3 (−9.6 to −3)	8	0.8 (−4.5 to 6.1)
MUFA diet in HFLC diet	11	1.9 (−3.9 to 7.7)	11	5.2 (−4.9 to 15.2)	11	21.0 (10.2–31.7)§	10	3.1 (−1.1 to 7.2)	10	−4.3 (−9.4 to 0.8)	7	2.8 (−3.4 to 8.9)
No MUFA diet in HFLC diet	11	−0.4 (−4.0 to 3.3)	11	11.8 (1.7–22)	11	9.4 (1.6–17.2)§	10	0.2 (−3.8 to 4.3)	10	−6.2 (−9.4 to −3)	9	−1.6 (−6.7 to 3.4)
WL diet in LFHC and HFLC diets	4	−2.1 (−9.6 to 5.5)	4	12.5 (−1 to 25.9)	4	4.0 (−7.1 to 15.2)‡	3	1.3 (−6 to 8.5)	3	−3.9 (−12.4 to 4.6)	3	1.9 (−7.4 to 11.2)
No WL diet in LFHC and HFLC diets	18	0.7 (−2.7 to 4.1)	18	6.9 (−1.5 to 15.3)	18	17.9 (10.2–25.5)‡	17	1.7 (−1.5 to 4.9)	17	−5.8 (−8.7 to −3)	13	−0.2 (−4.6 to 4.1)

*Studies having neither a washout period nor baseline data. †Parallel study design or cross-sectional design studies that have a washout period and/or baseline data. ‡$P < 0.01$; §$P < 0.05$. WL diet, energy intake restricted for weight loss.

tritional content must be appropriate for metabolic control regardless of macronutrient proportions (1).

Changes in FPG and A1C did not differ between the two diets despite significant elevations in 2-h and fasting insulin with the LFHC diet. One possible explanation is that the elevation in postprandial glucose level was overcompensated for by increased insulin secretion. However, only three studies concurrently assessed A1C, fasting insulin, and FPG values, with an intervention period of, at most, 6 weeks. Therefore, we could not conclude whether the elevation in postprandial glucose and insulin level achieved by raising the dietary C/F ratio leads to the deterioration of glycemic control represented by elevations in FPG and A1C.

A previous meta-analysis suggested that replacing carbohydrate with MUFA reduced fasting triglycerides in patients with type 2 diabetes on weight-maintenance diets (36); this was supported by our results. However, it is uncertain whether the effect on triglycerides was caused by the C/F ratio or the ratio of energy from MUFA to total energy. Moreover, whether the effect of this replacement was independent of that of a weight-loss diet has not been investigated. According to our stratified analyses, no dose-response relationship between the C/F ratio in the LFHC diet and the elevation in triglycerides was indicated, although replacement of the MUFA diet with the LFHC diet induced a greater elevation in triglycerides. Moreover, the LFHC diet did not significantly elevate triglycerides compared with the HFLC diet when a weight-loss diet was prescribed. Therefore, controlling total caloric intake and the quality of dietary fat appear to be more important than carbohydrate and fat composition in improving triglycerides levels. In other words, these findings suggest that a high-carbohydrate diet has little harmful effect on triglycerides levels if such a diet provides sufficient energy restriction for weight control.

Our study has some limitations. First, although we omitted studies investigating the effect of high-carbohydrate diets that were also high in dietary fiber, it is possible that the additional phytochemicals (including fiber itself), which are inevitably accompanied by a substantial amount of carbohydrate, influence the metabolic effects regardless of the change in C/F ratio. Second, we assumed that energy intake from the two diet groups would be similar if a weight-maintenance diet was equal to an isocaloric diet based on evidence of the meta-analysis by Bravata et al. (37) that indicated that weight change was associated with restriction of caloric intake but not reduced carbohydrate content. However, some recent studies showed that low-carbohydrate diets resulted in greater weight loss than low-fat diets despite their similar energy content (38), as is often the case with high-fiber diets (e.g., whole grains) (39). More investigation is needed to determine whether the relationship between change in energy intake and body weight is independent of the proportions of dietary carbohydrate and fat. Third, few studies investigated long-term effects (e.g., >2 months) of changing the proportions of carbohydrate and fat on metabolic profiles in patients with type 2 diabetes. Actually, a larger elevation in fasting insulin in association with the LFHC diet was observed for an intervention period of <4 weeks compared with ≥4 weeks but without statistical significance ($P = 0.10$). Possibly, a prolonged intervention involving changes in macronutrient composition causes some adaptation of insulin metabolism. Fourth, most studies provided insufficient data about baseline glucose and lipid levels, and few focused on black or Asian patients. Therefore, the current meta-analysis provides limited suggestions on identifying patients for whom a low-fat or low-carbohydrate diet is especially effective in terms of their circumstances or metabolic profiles (1).

Future studies focused on the following are suggested: 1) providing a possible explanation for the greater adverse effect on the fasting insulin by the LFHC diet than by the HFLC diet, especially in younger and leaner individuals; 2) identifying the long-term effect of a low-carbohydrate diet on factors other than metabolic effects (e.g., adaptation in glucose and lipid metabolism, ad libitum energy intake in patients with type 2 diabetes or obesity [40]) and the safety of such a diet (e.g., with regard to the digestive system); and 3) addressing whether a subject's medication status and the characteristics of diabetes drugs could influence the effect of changing the dietary C/F ratio in patients with type 2 diabetes.

In conclusion, replacement of dietary fat with carbohydrate is not recommended for improvement of insulin resistance in patients with type 2 diabetes under conditions whereby total energy and protein intake and the content of carbohydrate are similar and the proportion of carbohydrate to total energy is ≥30%. We found that younger and leaner patients had higher fasting insulin responses with the LFHC diet, although the biological mechanism was not fully investigated. The LFHC diet also adversely affects triglycerides and HDL cholesterol compared with the HFLC diet. However, energy restriction and dietary fat quality seemed more important for lowering the triglyceride concentration than the proportion of carbohydrate and fat.

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Polyunsaturated Fatty Acids Selectively Suppress Sterol Regulatory Element-binding Protein-1 through Proteolytic Processing and Autoloop Regulatory Circuit*

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Sterol regulatory element-binding protein (SREBP)-1 is a key transcription factor for the regulation of lipogenic enzyme genes in the liver. Polyunsaturated fatty acids (PUFA) selectively suppress hepatic SREBP-1, but molecular mechanisms remain largely unknown. To gain insight into this regulation, we established *in vivo* reporter assays to assess the activities of *Srebf1c* transcription and proteolytic processing. Using these *in vivo* reporter assays, we showed that the primary mechanism for PUFA suppression of SREBP-1 is at the proteolytic processing level and that this suppression in turn decreases the mRNA transcription through lowering SREBP-1 binding to the SREBP-binding element on the promoter ("autoloop regulatory circuit"), although liver X receptor, an activator for *Srebf1c* transcription, is not involved in this regulation by PUFA. The mechanisms for PUFA suppression of SREBP-1 confirm that the autoloop regulation for transcription is crucial for the nutritional regulation of triglyceride synthesis.

Polyunsaturated fatty acids (PUFA)[3] have been well established as negative regulators of hepatic lipogenesis (reviewed in Ref. 1). Allmann and Gibson (2) discovered that adding 2% linoleate to a high carbohydrate fat-free diet suppressed the rate of hepatic fatty acid biosynthesis and the activities of fatty-acid synthase and glucose-6-phosphate dehydrogenase by nearly 70% in mice. In contrast, supplementing the high carbohydrate diet with palmitate, oleate, or cholesterol had no effect on hepatic lipogenesis or the activity of lipogenic enzymes. Since then, a number of investigators have demonstrated that dietary PUFA of the *n*-6 and *n*-3 families suppress hepatic lipogenesis. This anti-lipogenic action of PUFA reflects decreases in mRNA levels of hepatic enzymes, including acetyl-CoA carboxylase, fatty-acid synthase, and stearoyl-CoA desaturase.

The fatty acid biosynthetic pathway, composed of some 25 enzymes, has been elucidated in detail (3). For the *de novo* synthesis of long chain saturated fatty acids, fatty-acid synthase, the main synthetic enzyme that catalyzes the condensation of malonyl-CoA to produce the 16-carbon saturated fatty acid palmitate, and acetyl-CoA carboxylase, which synthesizes malonyl-CoA from acetyl-CoA, are of particular importance. The regulation of these lipogenic enzymes has been revealed to be primarily controlled by a transcription factor sterol regulatory element-binding protein (SREBP)-1c (4, 5).

SREBPs are transcription factors that belong to the basic helix-loop-helix leucine zipper family and are considered to be profoundly involved in the transcriptional regulation of cholesterogenic and lipogenic enzymes (6, 7). Unlike other members of the basic helix-loop-helix leucine zipper family, SREBPs are synthesized as precursors bound to the endoplasmic reticulum and nuclear envelope. Upon activation, SREBPs are cleaved, and the N-terminal parts are released from the membrane into the nucleus as mature protein by a sequential two-step proteolytic processing. To date, three SREBP isoforms, SREBP-1a, -1c, and -2, have been identified and characterized. SREBP-1a and -1c are transcribed from the same gene, each by a distinct promoter, and the predominant SREBP-1 isoform in liver is 1c rather than 1a (8). It has been established by several lines of evidence, especially by those from transgenic and knock-out mouse models, that SREBP-1c controls hepatic lipogenesis, whereas SREBP-2 plays a crucial role in regulation of cholesterol synthesis (5, 9, 10).

In 1999, we and others reported that the PUFA-specific suppression of lipogenic enzymes is mediated by the reduction of nuclear SREBP-1c protein in the liver (11–14). Interestingly, PUFA selectively decreases SREBP-1, not affecting SREBP-2. The mechanism by which PUFA specifically suppresses SREBP-1c nuclear abundance, however, remains unclear, although several potential mechanisms have been implicated, including suppression of *Srebf1c* gene transcription

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[3] The abbreviations used are: PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; LXR, liver X receptor; LXRE, liver X receptor-binding element; SREBP, sterol regulatory element-binding protein; SRE, SREBP-binding element; pfu, plaque-forming unit; OLA, oleic acid ethyl ester; shRNA, short hairpin RNA; SCAP, SREBP cleavage-activating protein.

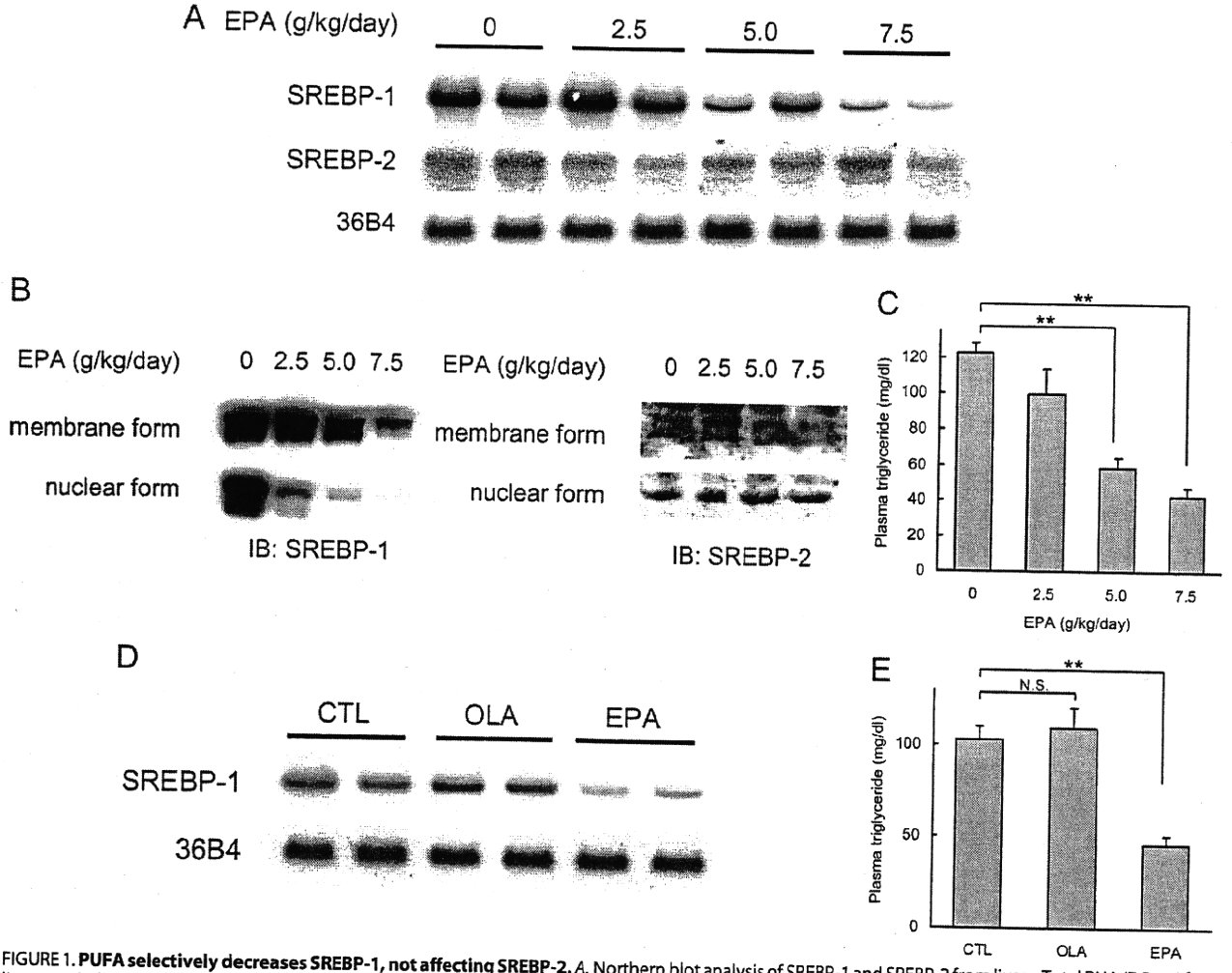


FIGURE 1. **PUFA selectively decreases SREBP-1, not affecting SREBP-2.** *A*, Northern blot analysis of SREBP-1 and SREBP-2 from livers. Total RNA (7.5 μg) from livers pooled equally from two mice for each group was subjected to Northern blotting to determine SREBP-1, SREBP-2, and 36B4 (used as a loading control) mRNA levels. ICR male mice were fed a high carbohydrate fat-free diet and treated orally with EPA at indicated doses once a day for 4 consecutive days. Control mice were treated orally with 7.5 g/kg water. Mice were sacrificed in a nonfasted state. *B*, immunoblot (*IB*) analysis of mature and precursor SREBP-1 and SREBP-2 proteins from livers. Aliquots of nuclear extracts (10 μg) and total proteins (50 μg) from livers pooled equally from four male mice for each group were subjected to immunoblot analysis. The primary antibodies used were polyclonal anti-mouse SREBP-1 and polyclonal anti-mouse SREBP-2. *C*, plasma triglycerides in EPA-treated mice. *D*, Northern blot analysis of SREBP-1 from livers. ICR male mice were fed a high carbohydrate fat-free diet and treated orally with 7.5 g/kg OLA or EPA once a day for 4 days. Total RNA (7.5 μg) from livers pooled equally from two mice for each group was subjected to Northern blotting to determine SREBP-1 and 36B4 (used as a loading control (*CTL*)) mRNA levels. *E*, plasma triglycerides in OLA- and EPA-treated mice. These data are representative of at least two independent experiments ($n = 4$ mice/group). Results are means ± S.E. **, $p < 0.01$; *N.S.*, not significant.

and proteolytic processing as well as enhancement of proteasomal degradation and mRNA decay (11, 15–18). As for the suppression of *Srebf1c* gene transcription by PUFA, we have previously identified liver X receptor-binding element (LXRE) and SREBP-binding element (SRE) on the *Srebf1c* promoter region by a series of promoter analyses (19, 20), and we have also suggested that PUFA can antagonize LXR in an *in vitro* setting (16).

These situations prompted us to clarify the molecular mechanism underlying the suppressive effect of PUFA on nuclear SREBP-1 abundance, especially in the *in vivo* setting. Because the inhibitory effect of fatty acids on SREBP-1 was specific and clear for PUFA in *in vivo* experiments, whereas many previous reports using *in vitro* system have failed to show this specificity (21, 22), we adopted an approach of *in vivo* reporter assays utilizing the *in vivo* imaging system (IVIS™; Xenogen, Alameda, CA). First, to examine the transcriptional mechanism, *in vivo* promoter analyses were performed, and the responsible cis-element on the *Srebf1c* promoter was located at SRE, not at LXRE. Next, the mechanism by which PUFA decreases the nuclear form of SREBP-1 was explored by another reporter system detecting proteolytic activity for the precursor form of SREBP-1, demonstrating that PUFA suppresses the maturation of SREBP-1 through proteolytic processes. From these experiments, we concluded that the primary mechanism for PUFA suppression of SREBP-1 expression is at the proteolytic processing level and that this suppression in turn decreases the *Srebf1c* mRNA transcription through lowering SREBP-1 binding to SRE on the promoter ("autoloop regulation" (19)).

EXPERIMENTAL PROCEDURES

Materials—Eicosapentaenoic acid (EPA) ethyl ester (95% grade) was provided from Mochida Pharmaceutical (Tokyo,

TABLE 1
Fatty acid composition in the liver
Fatty acid composition in the liver was analyzed by gas chromatography (n = 4 mice/group). Results are means ± S.E.

	EPA (g/kg/day)			
	0	2.5	5.0	7.5
	mg/g liver weight			
C12:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C14:0	0.22 ± 0.05	0.09 ± 0.03	0.05 ± 0.01[a]	0.07 ± 0.02[a]
C14:1*n*-5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C16:0	10.11 ± 1.07	8.06 ± 0.60	6.48 ± 0.40[a]	7.97 ± 0.58
C16:1*n*-7	2.06 ± 0.26	1.10 ± 0.18[a]	0.70 ± 0.04[b]	0.75 ± 0.13[b]
C18:0	3.12 ± 0.09	3.72 ± 0.17	3.13 ± 0.18	4.15 ± 0.18[b]
C18:1*n*-9	14.77 ± 2.96	7.76 ± 1.82[a]	3.70 ± 0.54[a]	4.88 ± 1.43[a]
C18:2*n*-6	1.76 ± 0.32	1.74 ± 0.23	1.92 ± 0.27	1.65 ± 0.30
C18:3*n*-6	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C18:3*n*-3	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
C20:0	0.04 ± 0.01	0.07 ±0.02	0.08 ± 0.02	0.08 ± 0.02
C20:1*n*-9	0.64 ± 0.17	0.22 ± 0.09	0.10 ± 0.02[a]	0.12 ± 0.04[a]
C20:2*n*-6	0.07 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00[a]
C20:3*n*-9	0.93 ± 0.06	0.18 ± 0.10[b]	0.07 ± 0.01[b]	0.08 ± 0.02[b]
C20:3*n*-6	0.44 ± 0.09	0.19 ± 0.02[a]	0.17 ± 0.03[a]	0.15 ± 0.01[a]
C20:4*n*-6	2.37 ± 0.33	1.79 ± 0.05	1.64 ± 0.15	1.54 ± 0.08[a]
C20:5*n*-3	0.12 ± 0.02	3.15 ± 0.31[b]	3.06 ± 0.17[b]	4.42 ± 0.35[b]
C22:0	0.09 ± 0.02	0.14 ± 0.03	0.16 ± 0.03	0.17 ± 0.04
C22:1*n*-9	0.04 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
C22:4*n*-6	0.11 ± 0.02	0.07 ± 0.01	0.06 ± 0.01[a]	0.06 ± 0.01[a]
C22:5*n*-3	0.05 ± 0.01	1.21 ± 0.33[a]	1.50 ± 0.23[b]	2.01 ± 0.44[b]
C22:6*n*-3	2.88 ± 0.23	3.60 ± 0.32	3.33 ± 0.34	3.65 ± 0.09[a]
C24:0	0.07 ± 0.01	0.10 ± 0.02	0.12 ± 0.01[a]	0.13 ± 0.02[a]
C24:1*n*-9	0.18 ± 0.01	0.18 ± 0.01	0.12 ± 0.02[a]	0.15 ± 0.01

[a] $p < 0.05$.
[b] $p < 0.01$.

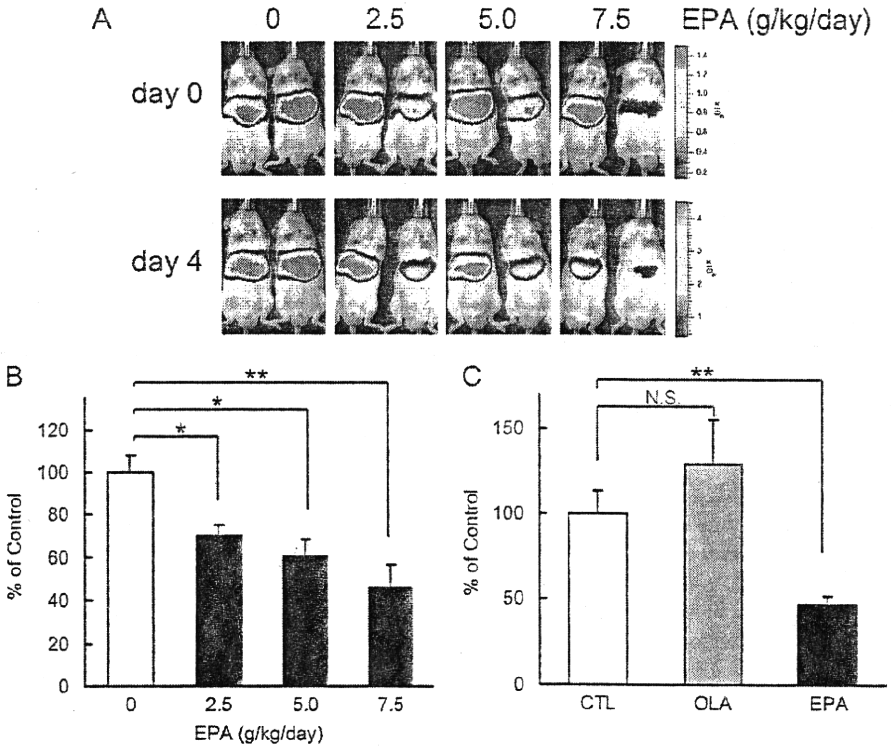


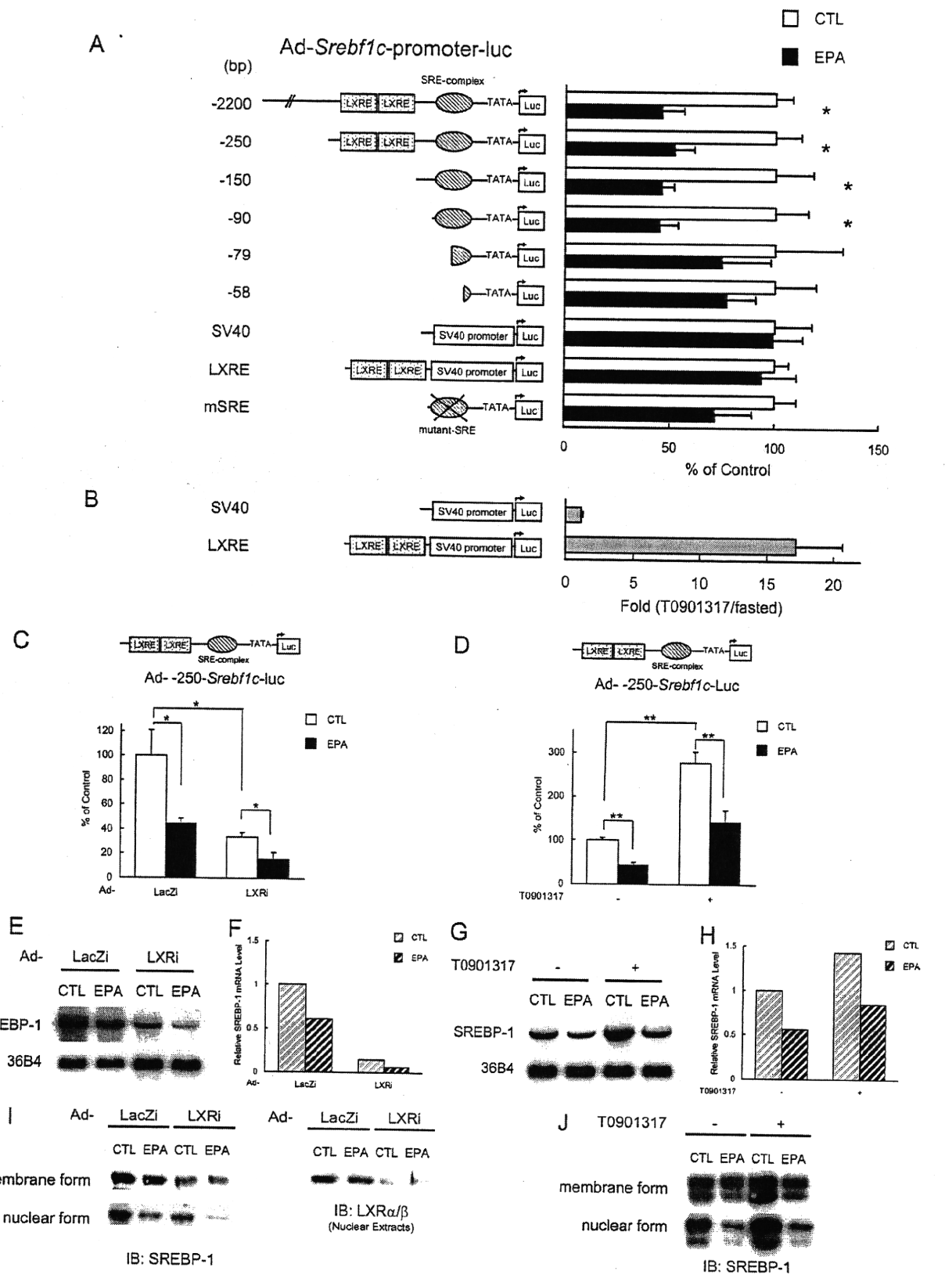
FIGURE 2. **PUFA suppresses *Srebf1c* promoter activity.** 2.2-kbp *Srebf1c*-Luc adenovirus (Ad-2.2k-*Srebf1c*-1c-Luc) (6.0×10^6 pfu/body) was intravenously injected into ICR male mice. After 4 days, mice (n = 4 for each group) were treated orally with EPA, OLA, or water (*CTL*) at indicated doses once a day for 4 days. *A*, on day 0 (before) and day 4 from the first treatment of EPA, luciferin was injected intraperitoneally in nonfasted mice, and the luminescence from liver was captured with IVIS. The *color overlay* on the image represents the photons/s emitted from the animal with a range of 1.5×10^5–1.5×10^6 photons/s (day 0), 5.0×10^5–5.0×10^6 photons/s (day 4), as indicated by the color scale next to the images. *B* and *C*, quantification of luciferase activity with LivingImage software. Fold changes of luciferase activity on day 4 *versus* day 0 are shown. These data are representative of at least two independent experiments (n = 4 mice/group). Results are means ± S.E. *, $p < 0.05$; **, $p < 0.01$, respectively. *N.S.*, not significant.

Japan) and GW532 (SCAP ligand) from GlaxoSmithKline (Les Ulis Cedex, France). The synthetic LXR agonist T0901317 was purchased from Cayman Chemical (Ann Arbor, MI). Standard laboratory diet (CRF-1, composed of 60% carbohydrate, 13% fat, and 27% protein on a caloric basis) and high carbohydrate fat-free diet (70% sucrose and 20% casein supplemented with methionine, vitamins, and minerals) were obtained from Oriental Yeast (Tokyo, Japan). Other materials were purchased from Sigma unless indicated otherwise.

Animals—Seven- to 9-week-old ICR male mice were purchased from CLEA (Tokyo, Japan). All animals were maintained in a temperature-controlled environment with a 12-h light/dark cycle and were given free access to standard laboratory diet and water. Four days before the start of indicated fatty acid administration, the basal diet was switched to a high carbohydrate fat-free diet. EPA or oleic acid ethyl ester (OLA) was administered orally once a day for 4 days. GW532 (0–15 mg/kg/day) or vehicle (0.9% carboxymethylcellulose, 9.95% polyethylene glycol 400, and 0.05% Tween 80) was administered orally once a day for 3 days. Mice were sacrificed in the light phase in a nonfasted state. All experiments were repeated at least twice. All animals studied were anesthetized and euthanized according to protocol approved by the Tokyo University Animal Care and Use Committee.

RNA Isolation and Northern Blotting—Total RNA from mouse liver was extracted using TRIzol reagent (Invitrogen), and a 7.5-μg RNA sample equally pooled among each group was run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. The cDNA probes for mouse SREBP-1, SREBP-2, and 36B4 were cloned as described previously (11). The probes were labeled with [α-^{32}P]dCTP using Megaprime DNA labeling system (Amersham Biosciences). The membranes were hybridized with the radiolabeled probe in RapidHyb Buffer (Amersham Biosciences) and washed in 0.1× SSC, 0.1% SDS at 65 °C. Blots were exposed to BAS imaging plate for the BAS2000 BIO imaging analyzer (Fuji Photo Film). The quantification results obtained with the BAS2000 system were normalized to the signal generated from 36B4 mRNA.

Nuclear Protein Extraction from Liver—Nuclear extract protein from mouse or rat liver was prepared as described previously (23). Briefly, excised livers (0.5 g) were homogenized in a Polytron in 5 ml of buffer A, which consisted of 10 mM HEPES, pH 7.9, 25 mM KCl, 1 mM EDTA, 2 M sucrose, 10% glycerol, 0.15 mM



spermine, and 2 mM spermidine, supplemented with protease inhibitors (6 μg/ml *N*-acetyl-leucyl-leucyl-norleucinal (ALLN, Calbiochem), 2.5 μg/ml pepstatin A, 2 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride and 2.5 μg/ml aprotinin). Pooled homogenate was then subjected to one stroke of a Teflon pestle in a Potter-Elvehjem homogenizer, followed by filtration through two layers of cheesecloth, and layered over 10 ml of buffer A. After centrifugation at 24,000 rpm on a Beckman SW28 rotor for 1 h at 4 °C, the resulting nuclear pellet was resuspended in a buffer containing 10 mM HEPES, pH 7.9, 100 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, and 10% glycerol supplemented with protease inhibitors, after which 0.1 volume of 5 M NaCl was added. Each mixture was agitated gently for 30 min at 4 °C and then centrifuged at 89,000 rpm on a Himac S120AT2 rotor (Hitachi, Tokyo, Japan) for 30 min at 4 °C. The supernatant was used as nuclear extract.

Immunoblotting of SREBP Proteins—Aliquots of nuclear extract (10 μg) and total lysate (50 μg) proteins were subjected to SDS-PAGE. Immunoblot analysis was performed using the ECL Western blotting detection system (Amersham Biosciences) and exposed to XAR-5 film (Eastman Kodak Co.). The primary antibodies for SREBPs (rabbit polyclonal; number 931 for mouse SREBP-1 and number 528 for SREBP-2) were used as described previously (24). The primary antibody for LXRα/β (H-144; sc-13068) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Fatty Acid Composition of Liver—Fatty acid composition was measured as described previously (24). An aliquot (0.1 g) of liver samples snap-frozen by liquid nitrogen was homogenized in 1 ml of normal saline. The fatty acid composition was measured by gas chromatography at Bio-Medical Laboratories (Tokyo, Japan) (25) Briefly, total lipids in liver homogenates were extracted according to the Folch's procedure (26), followed by transesterification of fatty acids with boron trifluoride/methanol at 100 °C for 90 min. The methylated fatty acids were then extracted with hexane and analyzed using a GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan) and BPX70 capillary column (0.25 mm inner diameter × 30 m, SGE International Ltd., Melbourne, Australia).

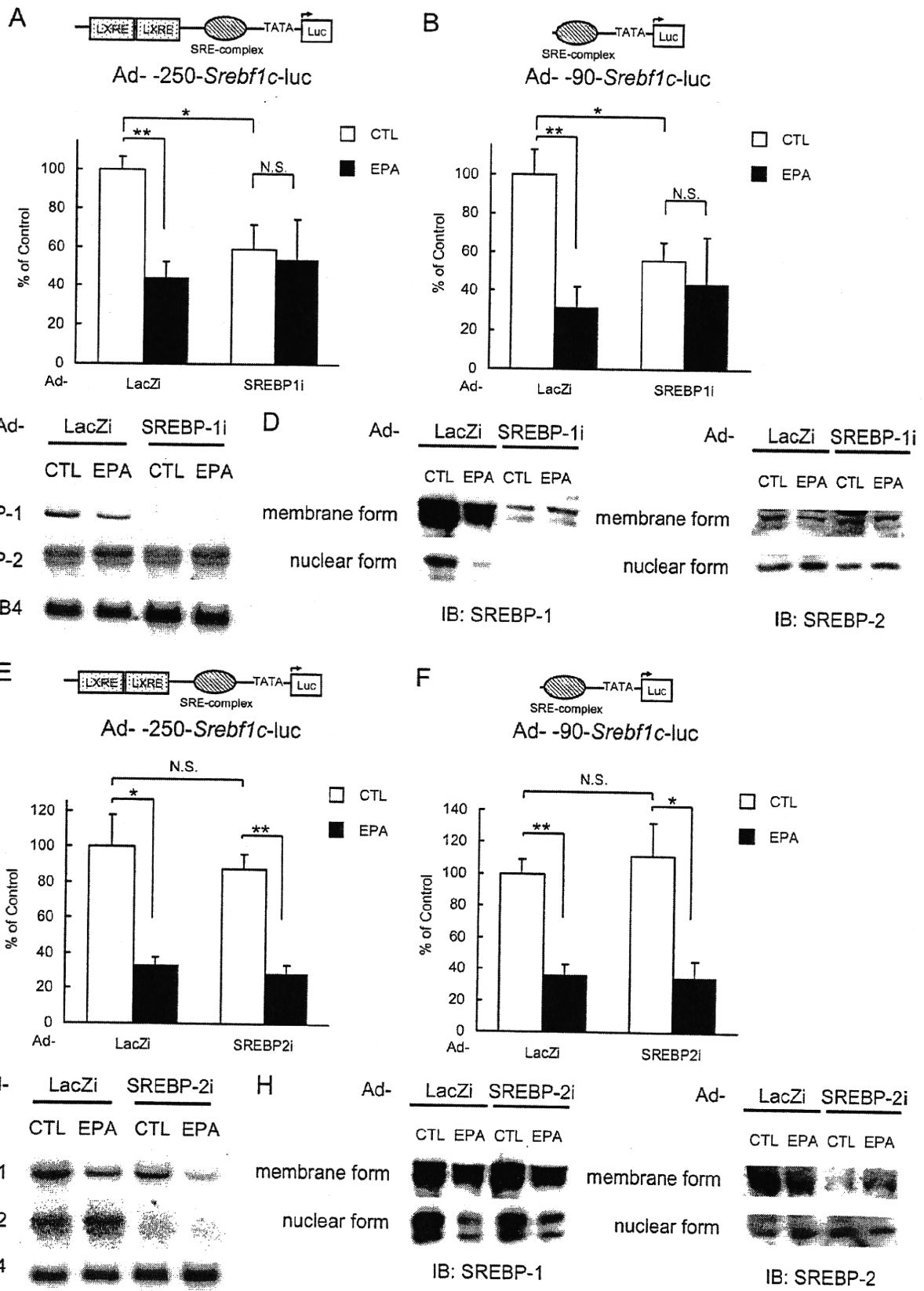
Transfection and Luciferase Assays—HEK293 cells were cultured in Dulbecco's modified Eagle's medium containing 25 mM glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate supplemented with 10% fetal bovine serum. For luciferase assay, HEK293 cells were seeded in a 48-well plate and incubated until 80% confluent. The indicated amounts of expression plasmids, firefly luciferase reporter plasmid, and pSV40-*Renilla* luciferase plasmid were co-transfected into HEK293 cells using SuperFect transfection reagent (Qiagen) according to the manufacturer's protocol. Total amounts of transfected DNA were adjusted with empty vector. The luciferase activity in transfectants was measured on a luminometer. *Renilla* luciferase activities were used to normalize transfection efficiencies.

In Vivo Imaging of Luciferase Activity—In vivo imaging was performed as described previously (27). Mice were anesthetized with isoflurane/oxygen, and 3.0 mg of luciferin dissolved in 0.4 ml of phosphate-buffered saline (7.5 mg/ml) was injected into the intraperitoneal cavity. Mice were imaged from the ventral side using an In Vivo Imaging System (IVIS™, Xenogen) 15 min following the injection of luciferin. Relative photon emission over the liver region was quantified using LivingImage™ software (Xenogen).

Plasmid Construction—To construct expression plasmids for GAL4-DNA binding domain (GAL4-DBD) and VP16-transactivation domain (VP16-AD) fused to human SREBPs, VP16-AD from pACT vector (Promega) was inserted into pM vector (Clontech) with various lengths of DNA fragment of human SREBP-1c (amino acids 1–1123 ("FL" for full length), 1–436 ("Nuc" for N-terminal nuclear part), or 431–1123 ("Reg" for C-terminal regulatory domain) or human SREBP-2 (amino acids 14–1141 (FL) or 450–1141 (Reg)) retrieved by PCR from pTK-HSV-hSREBP-1c, pTK-HSV-hSREBP-2, and pcDNA3.1(+)-SREBP-1c (28, 29). Gal4-RE-Luc plasmid was described previously (30). For the construction of an expression plasmid for mouse Insig-1, cDNA fragment was amplified by PCR with primers 5′-GGATCCATGCCCAGGCTGCACGACCACG-3′ and 5′-CTCGAGTCAGTCACTGTGAGGCTTTTCCG-3′ and cloned into pcDNA3 vector with hemagglutin tag at the N terminus using BamHI and XhoI. The expression plasmid for SCAP is a kind gift from Dr. Nakakuki.

Preparation of Recombinant Adenoviruses—To construct various lengths of mouse *Srebf1c* promoter luciferase reporter

FIGURE 3. **PUFA suppresses *Srebf1c* promoter activity through SRE site.** *A*, adenoviruses encoding various lengths of *Srebf1c* promoter, as well as two LXRE sites on *Srebf1c* promoter (−239 to −165) with SV40 promoter and SRE mutant version of 90-bp *Srebf1c* promoter, attached with luciferase (*Luc*) (6.0×10^6 pfu/body) were injected intravenously into ICR male mice. After 4 days, mice ($n = 4–7$ for each group) were treated orally with 7.5 g/kg EPA or water (*CTL*) once a day for 4 days. On day 0 and 4 after the first treatment of EPA, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. *B*, Ad-LXRE-Luc injected mice were administered orally with 50 mg/kg T0901317 or vehicle (0.9% carboxymethylcellulose, 9.95% polyethylene glycol 400, and 0.05% Tween 80) after fasting overnight. At 0 and 16 h following T0901317 treatment in fasted states, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. Fold change of luciferase activity at 16 *versus* 0 h was presented. *C*, *E*, *F*, and *I*, knockdown of hepatic LXRα/β by adenoviral expression of shRNA. 250-bp *Srebf1c*-Luc adenovirus (Ad-250-bp- *Srebf1c*-Luc; 6.0×10^6 pfu/body) plus adenovirus expressing LXRα/β-specific or LacZ-specific shRNA (Ad-LXRi or Ad-LacZi, respectively; 2.5×10^8 pfu/body) were intravenously injected into ICR male mice. After 4 days, the mice ($n = 4$ for each group) were treated orally with 7.5 g/kg EPA or water (*CTL*) once a day for 4 days. On day 0 and 4 after the first treatment of EPA, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. *D*, *G*, *H*, and *J*, inhibitory effect of PUFA on *Srebf1c* gene transcription is not affected by LXR agonist. 250-bp *Srebf1c*-Luc adenovirus (Ad-250bp- *Srebf1c*-Luc) (6.0×10^6 pfu/body) was intravenously injected into ICR male mice. After 6 days, the mice ($n = 4$ for each group) were treated orally with 7.5 g/kg EPA or water (*CTL*) once a day for 4 days. At day 3 of EPA treatment, the mice were administered orally with 10 mg/kg T0901317 or vehicle. On days 0 and 4 after the first treatment of EPA, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. *C* and *D*, quantification of luciferase activity with LivingImage software. Fold changes of luciferase activity on day 4 *versus* day 0 are shown. *E* and *G*, Northern blot analysis of SREBP-1 from livers. Total RNA (7.5 μg) from livers pooled equally from mice of each group was subjected to Northern blotting to determine SREBP-1 and 36B4 (used as a loading control) mRNA levels. *F* and *H*, quantification of the data shown in *E* and *G*. The fold change is the relative ratio of each signal *versus* the control mice. *I* and *J*, immunoblot (*IB*) analysis of mature and precursor SREBP-1 proteins and LXRα/β in livers. Aliquots of nuclear extracts (10 μg) and total proteins (50 μg) from livers pooled equally from four male mice of each group were subjected to immunoblot analysis. The primary antibodies used were polyclonal anti-mouse SREBP-1 and polyclonal anti-mouse LXRα/β. These data are representative of at least two independent experiments ($n = 4–7$ mice/group). Results are means ± S.E. *, $p < 0.05$, and **, $p < 0.01$ *versus* controls.



plasmids, DNA fragments retrieved from pGL2 vectors constructed previously (19, 20, 31) were inserted into pGL3 basic vector plasmids (Promega). The fragments including promoter region linked to luciferase reporter gene were inserted into the Gateway entry vector pENTR4 (Invitrogen) and generated by homologous recombination between the entry vector and the pAd promoterless vector (Invitrogen). The fragments of human SREBPs GAL4-DBD and VP16-AD fusion protein from various GAL4-SREBP vectors were inserted into pENTR4 and generated by homologous recombination with the pAd/CMV/V5-DEST vector (Invitrogen). Adenoviruses encoding SREBP-1-specific and LacZ-specific shRNA for RNA interference (SREBP1i and LacZi, respectively) were described previously (32). Adenovirus construct encoding SREBP-2-specific shRNA (SREBP2i) targeting 5′-GGAGCAGTCTCAACGTCAACG-3′ sequence on SREBP-2 was subcloned into U6 entry vector (Invitrogen) and generated by homologous recombination with the pAd promoterless vector. Adenovirus construct encoding both LXRα and LXRβ shRNA (LXRi) targeting 5′-ACAGCTCCCTGGCTTCCTA-3′ sequence on LXRα (33) and 5′-CTACAACCACGAGACAGAA-3′ sequence on LXRβ, respectively, was subcloned into U6 entry vector (Invitrogen) and generated by homologous recombination with the pAd promoterless vector. Recombinant adenoviruses were propagated in HEK293 cells and purified by CsCl gradient centrifugation as described previously (34).

RESULTS

PUFA Selectively Decreases SREBP-1, Not Affecting SREBP-2—In our first series of experiments, we verified the effects of PUFA on SREBP-1 and -2 expression in the liver. As expected, administration of EPA, one of the major *n*-3 PUFA in mammals, down-regulated SREBP-1 mRNA and protein expression (Fig. 1, *A, B, D,* and *E*), leading to a decrease in plasma triglycerides concentration (Fig. 1, *C* and *F*) as compared with control or oleate. The delivery of orally administered EPA to the liver was confirmed by gas chromatography analysis (Table 1). EPA administration did not affect the body weights of mice (data not shown). The dose-response relationship data suggested that the nuclear form is more sensitive to PUFA than the membrane form of protein or mRNA. In contrast, SREBP-2 mRNA and protein levels were not altered by PUFA.

PUFA Suppresses Srebf1c Promoter Activity through SRE Site—Next, we attempted to estimate the *Srebf1c* promoter activity in the liver with an *in vivo* luciferase reporter analysis. The luciferase reporter gene driven by the 2200-bp *Srebf1c* promoter was adenovirally transduced into mouse liver, and the transcriptional activity was assessed by measuring luciferase activity with the IVIS imaging system. The physiological activity of this promoter had previously been confirmed by transgenic mice (31). As shown in Fig. 2, EPA decreased the *Srebf1c* promoter activity in a dose-dependent manner.

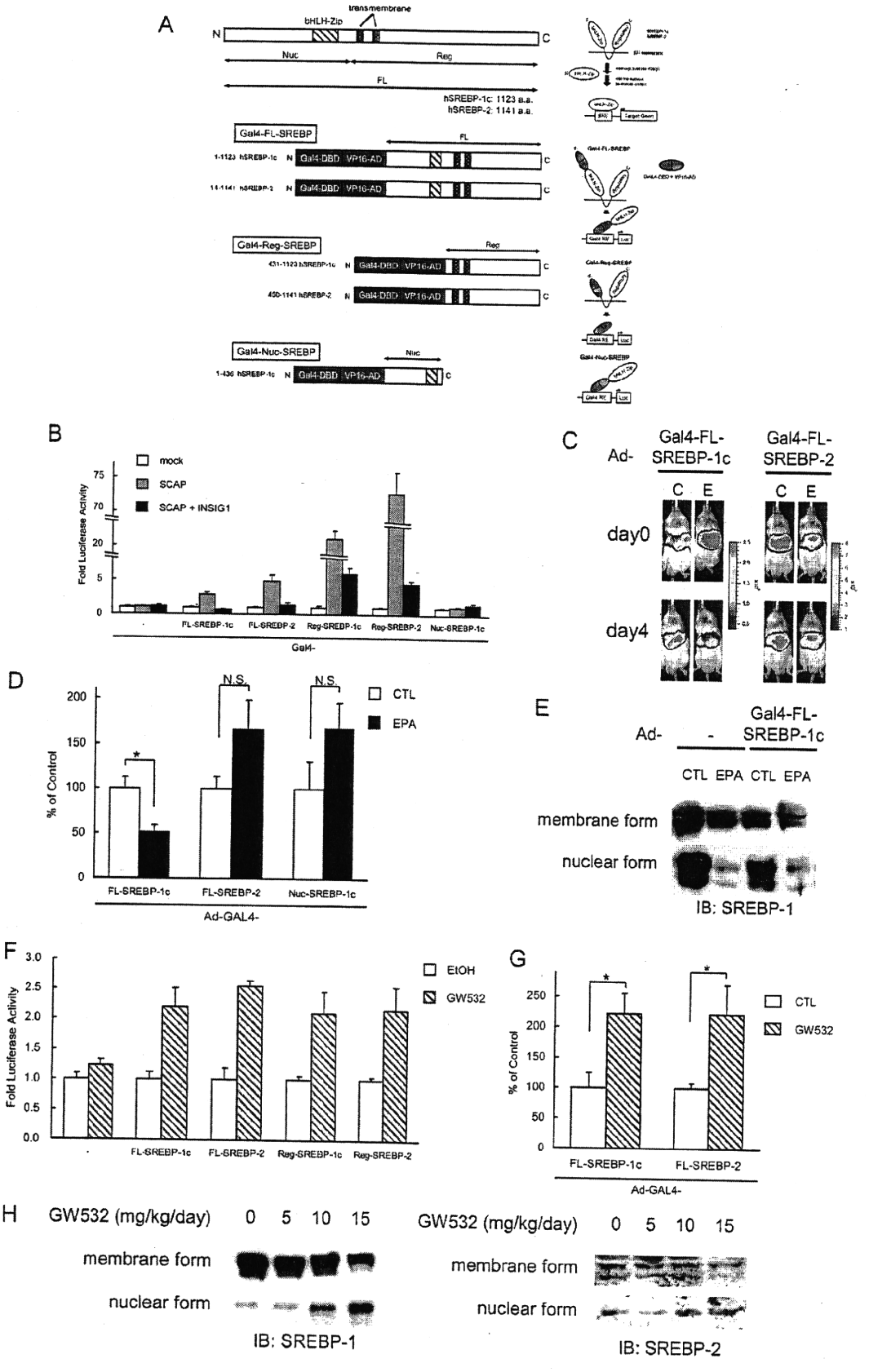
To determine the responsible region for PUFA suppression, a promoter deletion study was performed; six adenovirus constructs containing different lengths of the *Srebf1c* promoter (ranging from 2200 to 58 bp) were transduced into mouse livers, and the suppressive effect of EPA on promoter activity was assessed with IVIS (Fig. 3*A*). From this experiment, the responsible element was located at the SRE site within −90 to −60 bp upstream of the transcription start site. This result was confirmed by another experiment using a mutant SRE construct.

LXR Is Not Involved in the PUFA Suppression of Srebf1c Gene Expression in Vivo—Furthermore, it was clarified from the series of deletion studies that EPA did not suppress the promoter activity of a construct containing only LXRE (Fig. 3*A*), demonstrating that EPA does not antagonize LXR binding to LXRE on the *Srebf1c* promoter at least in the *in vivo* setting. Moreover, as shown in Fig. 3, *C, E, F,* and *I*, it was demonstrated that the simultaneous knockdown of both LXRα and -β did not affect the suppressive effect of PUFA on *Srebf1c* promoter activity. Furthermore, the stimulation of LXR by an LXR agonist T0901317 was also shown to be independent of the inhibitory effect of PUFA, as shown in Fig. 3, *D, G, H,* and *J*. Based on these findings, we concluded that LXR is not the direct target of PUFA regulation in the *in vivo* setting, although LXR is a determinant of the expression level of SREBP-1 mRNA.

Inhibitory Effect of PUFA on Srebf1c Gene Transcription Is Mediated through SREBP-1 Itself—To determine whether the trans-acting factor for SRE is SREBP-1 or -2 or both, SREBP-1/2 was knocked down by RNA interference. As shown in Fig. 4, *A* and *B*, the effects of PUFA administration and knocking down SREBP-1 were essentially equal, and when SREBP-1 was knocked down, the promoter activity of *Srebf1c* gene was reduced by half, and PUFA showed no additive effects. In contrast, knocking down SREBP-2 did not affect the promoter activity of *Srebf1c* as assessed by luciferase reporter expression (Fig. 4, *E* and *F*), demonstrating that SREBP-2 is not involved in the transcriptional regulation of *Srebf1c* gene expression. From these findings, it was concluded that the trans-acting factor for SRE on the *Srebf1c* promoter is SREBP-1 and not SREBP-2. This indicates that SREBP-1 constitutes an autoloop regulatory circuit.

PUFA Suppresses the Proteolytic Activation of SREBP-1, Not Affecting SREBP-2—SREBP-1 is synthesized as a precursor bound to the endoplasmic reticulum and nuclear envelope

FIGURE 4. **Inhibitory effect of PUFA on *Srebf1c* gene transcription is mediated through SREBP-1 itself.** *A–H,* knockdown of hepatic SREBP-1 (*A–D*) or SREBP-2 (*E–H*) by adenoviral expression of shRNA. 250- or 90-bp *Srebf1c*-Luc adenovirus (Ad-250bp- *Srebf1c*-Luc or Ad-90bp- *Srebf1c*-Luc; 6.0×10^6 pfu/body) plus adenovirus expressing SREBP-1-specific, SREBP-2-specific, or LacZ-specific shRNA (Ad-SREBP1i, Ad-SREBP2i, or Ad-LacZi; 2.5×10^8 pfu/body) were intravenously injected into ICR male mice. After 4 days, the mice ($n = 3–6$ for each group) were treated orally with 7.5 g/kg EPA or water (*CTL*) once a day for 4 days. On days 0 and 4 after the first treatment of EPA, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. *A, B, E,* and *F,* quantification of luciferase activity with LivingImage software. Fold changes of luciferase activity on day 4 *versus* day 0 are shown. *C* and *G,* Northern blot analysis of SREBP-1 and -2 in the liver. Total RNA (7.5 μg) from livers pooled equally from mice for each group was subjected to Northern blotting to determine SREBP-1, -2, and 36B4 (used as a loading control) mRNA levels. *D* and *H,* immunoblot (*IB*) analysis of mature and precursor SREBP-1 and -2 proteins in the liver. Aliquots of nuclear extracts (10 μg) and total proteins (50 μg) from livers pooled equally from four male mice of each group were subjected to immunoblot analysis. The primary antibodies used were polyclonal anti-mouse SREBP-1 and polyclonal anti-mouse SREBP-2. These data are representative of at least two independent experiments ($n = 3–6$ mice/group). Results are means ± S.E. *, $p < 0.05$, and **, $p < 0.01$, respectively.



and is released from the membrane into the nucleus as a mature protein by a cleavage process (7). To explore the molecular mechanism by which PUFA decreases the nuclear form of SREBP-1, another reporter system to detect proteolytic activity for the precursor form of SREBPs was constructed (Fig. 5*A*). In this system, Gal4-DNA binding domain and VP16 activation domain were attached to the N terminus of SREBPs, and the nuclear entry of this N-terminal fragment was measured by the Gal4-UAS system to assess the proteolytic release of the SREBP N termini. Using this cleavage-detecting reporter system, the proteolytic activities for precursor forms of SREBP-1 or -2 were tested *in vitro* with SCAP and insulin-induced gene (INSIG)-1 overexpressions as an activator and an inhibitor for cleavage of SREBP, respectively. As expected, the SCAP overexpression drastically enhanced the cleavage of N-terminal fragments of both SREBP-1 and -2, and the co-expression of INSIG1 reversed the effect of SCAP overexpression (Fig. 5*B*), indicating that this reporter system reflects the physiological regulation of SREBP activation processes by proteolysis. Next, we transduced the reporter genes into mouse livers using adenoviruses and examined the effect of PUFA in the *in vivo* setting. As shown in Fig. 5, *C* and *D*, EPA suppressed only the SREBP-1 cleavage-detecting reporter but did not affect the SREBP-2 reporter. Additionally, EPA did not decrease the reporter activity from the construct that contains only the N terminus (designated as Nuc-SREBP-1c) and enters the nucleus without cleavage, demonstrating that EPA did not accelerate the degradation of reporter fragment. In contrast, GW532, a SCAP activator, enhanced the cleavage of both SREBP-1 and -2 in the *in vitro* (Fig. 5*F*) and *in vivo* (Fig. 5*H*) situations.

DISCUSSION

This study has clearly demonstrated that the primary mechanism of the inhibitory effect of PUFA is the suppression of the proteolytic activation of SREBP-1 and that the transcriptional regulation is secondary to this post-translational suppression of mature SREBP-1 that itself binds to the SRE site on the *Srebf1c* promoter (autoloop regulation (19)), whereas LXR is not involved in the transcriptional regulation by PUFA (Fig. 6).

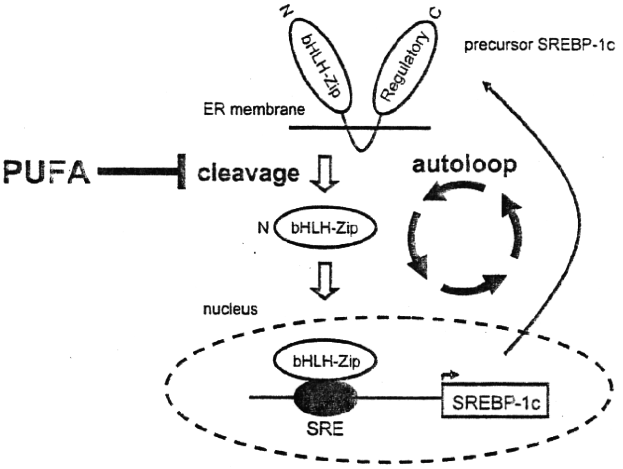


FIGURE 6. **Schematic representation of molecular mechanisms for inhibitory effects of PUFA on SREBP-1.** The primary mechanism for PUFA suppression of SREBP-1 is at the proteolytic processing level, and this suppression in turn decreases the mRNA transcription through lowering SREBP-1 binding to SRE on the promoter (autoloop regulatory circuit), although LXR is not involved in this regulation.

This is the first report that has clearly demonstrated the inhibitory effect of PUFA on the SREBP-1 proteolytic processing *in vivo*. This result is consistent with our previous report showing that PUFA decreases nuclear SREBP-1, although it does not affect the nuclear abundance of the truncated form of SREBP-1 expressed from a transgene, suggesting that PUFA

FIGURE 5. **PUFA suppresses the proteolytic activation of SREBP-1, not affecting SREBP-2.** *A*, various regions of human SREBP-1c (amino acids (*a.a.*) 1–1123 (FL), 431–1123 (Reg), 1–436 (Nuc)) and human SREBP-2 (amino acids 14–1141 (FL), 450–1141 (Reg)) were fused to Gal4-DNA binding domain and VP16-transactivation domain. *FL*, full length; *Nuc*, nuclear; *Reg*, regulatory; *bHLH-Zip*, basic helix-loop-helix leucine zipper. GAL4-VP16-SREBP fusion protein is attached to the endoplasmic reticulum (*ER*) similarly as endogenous SREBP protein. Upon activation, its N-terminal region is released from the membrane into the nucleus by a cleavage process. The GAL4-VP16 promotes the luciferase (*Luc*) reporter gene expression by binding to GAL4-RE containing eight copies of upstream activation sequence, Gal4-binding site. *B*, regulation of cleavage of GAL4-VP16 fusion SREBP protein by SCAP and INSIG-1. HEK293 cells in a 48-well plate were co-transfected with GAL4-RE-Luc plasmid (100 ng/well), expression plasmids of GAL4-VP16 fusion SREBP protein (100 ng/well), SCAP (250 ng/well), INSIG-1 (100 ng/well), and pSV40-Renilla plasmid (50 ng/well). Cells were harvested 24 h after transfection. The firefly luciferase activity was measured and normalized by *Renilla* luciferase activity. All experiments were performed in triplicate. *C–E*, regulation of cleavage of GAL4-VP16 fusion SREBP protein by EPA *in vivo*. GAL4-RE-Luc adenovirus (Ad-GAL4-RE-Luc, 2.0×10^8 pfu/body) plus adenovirus expressing GAL4-VP16 fusion SREBP protein (Ad-Gal4-FL-SREBP-1c, Ad-Gal4-FL-SREBP-2 or Ad-Gal4-Nuc-SREBP-1c, 1.0×10^8 pfu/body) were intravenously injected into ICR male mice. After 2 days, the mice ($n = 7–9$ for each group) were treated orally with 7.5 g/kg EPA or water (*CTL*) once a day for 4 days. *C*, 0 and 4 days after the fast treatment of EPA, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. The color overlay on the image represents the photons/s emitted from the animal with a range of 4.0×10^5–2.5×10^6 photons/s (Ad-Gal4-FL-SREBP-1c) and 1.0×10^6–8.0×10^6 photons/s (Ad-Gal4-FL-SREBP-2), as indicated by the *color scale* next to the images. *D*, quantification of luciferase activity with LivingImage software. Fold changes of luciferase activity on day 4 *versus* day 0 are shown. *E*, immunoblot analysis of mature and precursor SREBP-1 proteins from livers. Aliquots of nuclear extracts (10 μg) and total proteins (50 μg) from livers pooled equally from male mice for each group were subjected to immunoblot analysis. The primary antibodies used were polyclonal anti-mouse SREBP-1. *N.S.*, not significant. *F–H*, SCAP ligand GW532 accelerates SREBP-1 cleavage. *F*, regulation of cleavage of GAL4-VP16 fusion SREBP protein by GW532. HEK293 cells in a 48-well plate were co-transfected with GAL4-RE-Luc plasmid (100 ng/well), expression plasmids for GAL4-VP16 fusion SREBP protein (100 ng/well), and pSV40-*Renilla* plasmid (50 ng/well). 3 h after transfection, GW532 (1 μM) or EtOH was added to media, and cells were harvested 24 h after transfection. The firefly luciferase activity was measured and normalized by *Renilla* luciferase activity. All experiments were performed in triplicate. *G* and *H*, GAL4-RE-Luc adenovirus (Ad-GAL4-RE-Luc, 2.0×10^8 pfu/body) plus adenovirus expressing GAL4-VP16 fusion SREBPs protein (Ad-Gal4-FL-SREBP-1c or Ad-Gal4-FL-SREBP-2, 1.0×10^8 pfu/body) were intravenously injected into ICR male mice. After 2 days, the mice ($n = 8$ for each group) were administered orally with GW532 or vehicle at indicated doses. 6 h after the last treatment, luciferin was injected intraperitoneally, and the luminescence from liver was captured with IVIS. *G*, quantification of luciferase activity with LivingImage software. Fold changes on day 3 *versus* day 0 are shown. *H*, immunoblot analysis of mature and precursor SREBP-1 and SREBP-2 proteins from livers. Aliquots of nuclear extracts (10 μg) and total proteins (50 μg) from livers pooled equally from four male mice for each group were subjected to immunoblot (*IB*) analysis. The primary antibodies used were polyclonal anti-mouse SREBP-1 and anti-mouse SREBP-2. These data are representative of at least two independent experiments ($n = 3–8$ mice/group). Results are means ± S.E. *, $p < 0.05$.