Expression of Retinoic Acid, Triiodothyronine, and Glucocorticoid Hormone Nuclear Receptors Is Decreased in the Liver of Rats Fed a Hypercholesterolemia-Inducing Diet

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Several studies have shown that dietary factors modulate cell signaling pathways. The aim of this study was to determine whether a hypercholesterolemia-inducing diet rich in saturated fat and cholesterol modifies rat liver expression of the nuclear receptors of retinoic acid (RAR), triiodothyronine (TR), and glucocorticoid hormone (GR), which are transcriptional factors. The experimental diet contained coconut oil 25 g/100 g as a source of lipids, cholesterol 1 g/100 g, and cholic acid 0.5 g/100 g, and the control diet contained olive oil 5 g/100 g. After 26 days of feeding the hypercholesterolemia-inducing diet, a lower binding capacity of the nuclear receptors and a smaller amount of their mRNA were observed. Moreover, the activities of malic enzyme (ME) and tyrosine aminotransferase (TAT), whose gene promotors contain a response element to TR and GR, respectively, were significantly decreased. These changes occurred in a cellular environment characterized by a high level of cholesterol and free fatty acids (FFAs). Thus, two nonexclusive hypotheses can be proposed to explain this decreased expression of nuclear receptors, one emphasizing the effect of lipidic components on the cellular amount of receptor ligands (retinoic acid [RA] and triiodothyronine [T_3]), the other emphasizing a modification of the balance between nuclear receptors that could impede the upregulation of TR and RAR.

DIETARY FATS, particularly saturated fat and cholesterol, have received considerable attention because of their linkage to several chronic diseases including obesity, atherosclerosis, and certain types of cancer. Among the biomolecular mechanisms responsible for the onset of these diseases, changes in signal transduction pathways have been evoked, but it can be hypothesized that there are also changes in cell signaling pathways involving hormones acting at the nuclear level.

Retinoids, as well as steroid and thyroid hormones, are small lipophilic molecules that affect cellular differentiation and homeostasis through nuclear receptors belonging to the superfamily of nuclear hormone receptors. These receptors (retinoic acid [RA] receptor [RAR], 9-*cis* RAR [RXR], triiodothyronine [T_3] receptor [TR], and glucocorticoid hormone receptor [GR]), which function as ligand-dependent transcription factors, interact with polymorphic response elements linked to target genes. The complexity of the signaling pathway of these hormones results from the ability of nuclear receptors (at least TR and RAR) to form heterodimers with the common partner RXR, on one hand, and from the possible modulation of the binding properties of these receptors by hormonal and nutritional factors, on other hand.

In recent years, attention has been focused on a cohort of molecules derived from endogenous metabolism and/or nutrition as potential regulators of cell signaling pathways. Thus, a high level of dietary fat induces a lipid status characterized by alteration of fatty acid (FA) and cholesterol profiles that, in turn, affects a number of cellular functions including hormone binding and signal transduction mechanisms. However, the respective involvement of FAs and cholesterol remains to be determined. Many studies have shown the positive or negative effect of FAs on the binding of glucocorticoid hormone with GR,[1,2] or the inhibiting effect of FAs on the binding of T_3 with TR.[3-5] On the other hand, no information is available regarding the effect of cholesterol on the binding properties of TR, RAR, or GR.

This study was thus designed to explore the effect of a cholesterol-containing diet high in saturated FAs (SFAs) on the expression of these receptors in rat liver. Receptor expression was evaluated as the binding properties of RAR, TR, and GR, quantitation of the mRNA for these receptors, and an assay of the activity of enzymes with genes containing response elements for these receptors in their promoters: tissue transglutaminase (tTG),[6] malic enzyme (ME),[7] and tyrosine aminotransferase (TAT),[8] respectively.

MATERIALS AND METHODS

Experimental Design

The official French regulations (order no. 87-848) for the care and use of laboratory animals were followed. Male Wistar rats weighing about 190 g were obtained from the Center of Applied Pharmacology (Pamplona, Spain) and reared in the Department of Physiology and Nutrition (University of Navarra, Pamplona, Spain). Animals were housed in groups of four under a controlled environment (22° to 24°C and 12-hour light/dark cycle) with food and water freely available. Rats were acclimated for 5 days and had free access to water and a control diet. After the acclimation period, the rats were divided randomly into two groups of 10. One group was fed the control diet, and the other group was fed a diet containing cholesterol (1%) and cholic acid (0.5%) and high in SFA (25%) (Tables 1 and 2), which is known to induce a hypercholesterolemic status.[9,10] Body weight was recorded twice per week. Rats were killed by decapitation (between 9 and 11 AM) 26 days after the start of dietary treatment. Blood was collected, and the liver

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Table 1. Composition of the Experimental Diets (g/kg)

Component	Control Diet	Hypercholesterolemia-Inducing Diet*
Casein	200	200
DL-Methionine	4	4
Wheat starch	324	—
Sucrose	324	484
Cellulose	50	—
Olive oil	50	—
Coconut oil	—	250
Cholesterol	—	10
Cholic acid	—	5
Choline	2	2
AIN-76A mineral mixture†	35	35
AIN-76A vitamin mixture†	10	10

*According to Mengheri et al[9] and Jimenez et al.[10]
†Supplied by Dyets (Bethlehem, PA).

was rapidly excised and washed (twice) in cold saline (NaCl 9 g/L) solution. Portions of the liver were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

Binding Properties of Receptors in Rat Liver

The binding properties of receptors (maximum binding capacity and K_d) were derived from Scatchard analysis of results obtained by in vitro binding studies. Scatchard curves were drawn using linear regression analysis of the data (Sigma Plot Scientific Graphing System 4.02; Jandel, Corte Madera, CA).

Isolation of Liver Nuclei

All tissue fractionations were performed at 4°C. Nuclei were prepared according to the method of DeGroot and Torresani.[11]

RAR Binding

Due to the sensitivity of RA to numerous physicochemical factors (particularly light and oxygen) and its rapid degradation by enzymes contained in tissue extracts, a synthetic analog of RA was used as a ligand. This analog, 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl) benzoic acid (CD367), was synthesized and tritiated at the Centre International de Recherche Dermatologique (CIRD Galderma, Sophia Antipolis, Valbonne, France). CD367 behaves as a nonselective high-affinity ligand for the three types of RARs (RARα, RARβ, and RARγ).[12] Previous experiments performed in our laboratory have indeed shown that CD367 can be validly used to study binding of the RAR in rat liver.[13] Briefly, nuclear extracts were obtained by submitting nuclear pellets to DNase I digestion and salt extraction. Binding assays were performed according to the method of Daly et al.[14] Incubations with CD367 (12.5 to 125 nmol/L, 0.132 mCi/μg) were performed at 4°C for 1 hour. Nonspecific binding was estimated in parallel assays to which an approximately 1,000-fold excess of unlabeled CD367 was added. Bound and free CD367 were separated by high-performance size-exclusion chromatography on TSK gel G3000SW (CIL Cluzeau, Puteaux, France).

Table 2. FA Composition of Dietary Lipids (mg/g diet)

Component	Control Diet	Hypercholesterolemia-Inducing Diet
FAs		
8:0		19.0
10:0		14.3
12:0		112.7
14:0		42.8
16:0	5.4	21.5
18:0	1.2	6.0
20:0	0.2	
16:1(n-7)	0.8	
18:1(n-9)	35.8	16.8
18:2(n-6)	4.0	3.5
18:3(n-3)	0.5	
ΣSFA	6.8	216.3
ΣMUFA	36.6	16.8
ΣPUFA	4.5	3.5

NOTE. Composition was calculated according to the formulation of the diets (Table 1) and using food composition tables.

TR Binding

The TR site concentration and affinity for T_3 were estimated in saturation experiments described by Torresani and DeGroot.[15] Nuclear extracts were obtained using 400 mmol/L KCl in 20 mmol/L Tris hydrochloride, pH 7.9, and 1 mmol/L $MgCl_2$. Incubations with ^{125}I-T_3 (0.03 to 0.6 nmol/L, 3 mCi/μg) were performed at 20°C for 3 hours. Nonspecific binding was estimated in parallel assays to which an approximately 1,000-fold excess of radioinert T_3 was added. Bound and free T_3 were separated using a Dowex 1X8 anion-exchange resin (Sigma, St Quentin Fallavier, France) that binds free T_3.

GRn (GR located in nucleus) Binding

GRn binding was performed as previously described by Kaufman and Shaper[16] with slight modifications. Nuclei were suspended in STM buffer (250 mmol/L sucrose, 50 mmol/L Tris hydrochloride, pH 7.4 at 4°C, and 5 mmol/L $MgSO_4$). Duplicate 0.1-mL aliquots of this suspension (containing 300 μg protein) were incubated overnight (16 to 20 hours) at 4°C with a range of [^{3}H]dexamethasone (96 μCi/μg) concentrations (10 to 100 nmol/L) either alone or in the presence of a 1,000-fold excess of unlabeled dexamethasone to determine nonspecific binding. After incubation, samples were diluted with 2 mL STM buffer and centrifuged at 1,300 × *g* for 15 minutes. The pellets were washed three times with 3 mL STM buffer to remove unbound hormone. Then, samples were extracted with 1 mL ethanol at 22°C for a minimum of 30 minutes, cooled to 4°C, and sedimented at 1,300 × *g* for 15 minutes. Aliquots of supernatant were counted in a liquid scintillation counter.

Quantitation of mRNAs

The mRNAs were quantified using a semiquantitative method of reverse transcription and amplification by the reverse transcriptase–polymerase chain reaction (RT-PCR). Values for RAR, TR, and GR mRNAs were obtained by comparison against an internal standard, β-actin, that was simultaneously reverse-transcribed and amplified in the same test tube. Indeed, β-actin is known to be insensitive to nutritional and hormonal conditions.[17] Moreover, using a competitive RT-PCR method and the PCR MIMIC Construction Kit (Clontech Laboratories, Palo Alto, CA), we verified that the diet used had no effect on the hepatic concentration of β-actin mRNA.

Extraction of RNA was performed according to the method of Chomczynski and Sacchi[18] (modified). A portion of rat liver was homogenized in extraction buffer (3:1 vol/vol, 5.3 mol/L guanidium thiocyanate, 0.2 mol/L Trishydrochloride pH 7.5, and 0.04 mol/L EDTA: solution of 2% dithiothreitol-*N*-lauryl sarcosin), and total RNA was subsequently extracted from this homogenate with an equal volume of phenol:chloroform:isoamylic alcohol (49:49:2).

The position and sequence of the different oligonucleotide primers are summarized in Table 3. The primers for TR mRNA were chosen to exclusively quantify mRNA encoding for proteins that bind T_3 (TRα1 and TRβ1), and thus it was possible to compare the mRNA abundance with the binding capacity of receptors. Concerning RAR mRNA,

Table 3. Sequence of Oligonucleotide Primers and Size of Amplified Fragments

Primer	Sequence	Complementary Site	Size of Amplified Fragment (bp)
β-Actin*			
A1	AGGATGCAGAAGGAGATTACTGCC	2814-2837	222
A2	GTAAAACGCAGCTCAGTAACAGTCC	3159-3135	
RAR†			
R1	CTCACTGAGAAGATCCGGAAAGCCCACC	538-565	143
R2	TTGGTGGCCAGCTCACTGAATTTGTCCC	680-653	
TR‡			
T1	TCCTGATGAAGGTGACGGACCTGC	1247-1270	118
T2	TCAAAGACTTCCAAGAAGAGAGGC	1364-1341	
GR§			
G1	TGAGACCAGATGTAAGCTCTCCTC	1321-1344	167
G2	AATTGTGCTGTCCTTCCACTGCTC	1488-1465	

NOTE. Primers A1 and A2 were chosen in 2 different exons; the size of the PCR product provided a check that the amplified fragment was not derived from genomic DNA.

*From rat cytoplasmic β-actin gene according to the sequence in Nudel et al.[19]

†From murine RAR cDNA according to the sequence in Zelent et al.[20]

‡From rat TR cDNA according to the sequence in Murray et al.[21]

§From rat GR cDNA according to the sequence in Miesfield et al.[22]

primers were chosen to exclusively quantify mRNA encoding for RARβ, since this subtype is abundant in rat liver[23] and subject to nutritional regulation.[24] Primers were purchased from GENSET (Paris, France).

The cDNA preparation, PCR analysis, and quantitative determination of PCR products were performed as previously described.[25] Figure 1 shows, for example, the results of electrophoresis of PCR products of rat transcripts of β-actin, TR, RAR, and GR genes and a semilogarith-

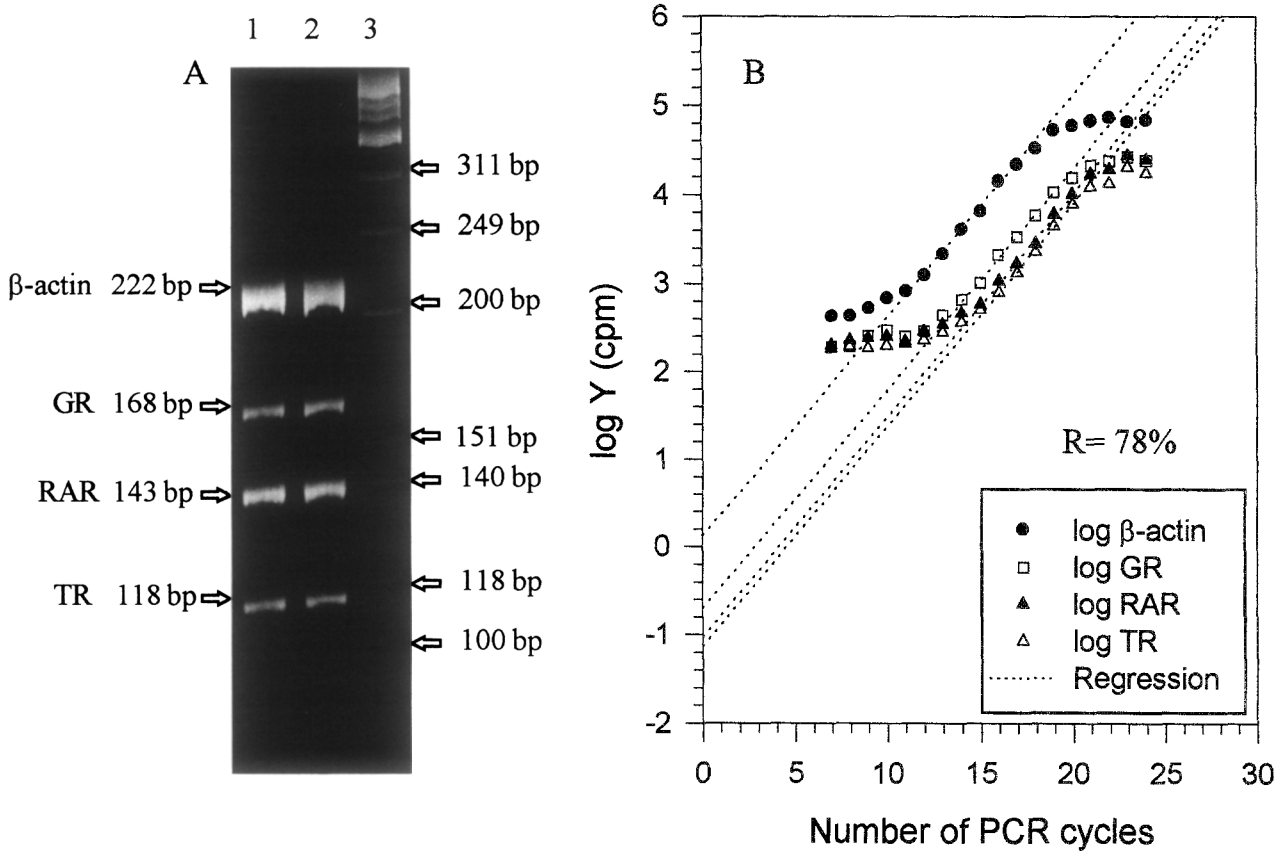

Fig 1. Relative quantification of GR, RAR, and TR transcripts in rat liver. (A) RT-PCR products resolved on 10% acrylamide gel electrophoresis and stained with ethidium bromide. Lanes 1 and 2, coamplification of β-actin, GR, RAR, and TR transcripts in rat liver; lane 3, molecular size markers ϕX174/*HinfI*. (B) Semilogarithmic representation of the relative amplification (Y) measured by counting the amount of [α^{32}P]dCTP incorporated. R, PCR efficiency.

mic representation of the relative amplification measured by counting the amount of ^{32}P incorporated.

Free FA Assay

Lipids from liver homogenates obtained at 4°C were extracted with chloroform:methanol (2:1 vol/vol).[26] Free FAs (FFAs) were isolated by thin-layer chromatography on silica gel 60H (Merck, Darmstadt, Germany) using hexane:ether:acetic acid (90:10:1 vol/vol) as the developing solvent. The FFA fraction was scraped from the plates, and methyl esters were made by esterification of FFAs in boron fluoride methanol at 100°C for 10 minutes.[27] Methyl esters were extracted and analyzed by gas-liquid chromatography on a Carlo Erba (Milan, Italy) chromatograph equipped with a hydrogen flame ionization detector). Separations were performed on a CP Sil 88 fused-silica capillary column (50 m × 0.25 mm ID, 0.20-μm film; Chrompack, Les Ulis, France). The column was programed at 150°C, and then the temperature was increased at a rate of 5°C/min up to 225°C and maintained at this point for 10 minutes. The temperature of the detector and the injector was 250°C, and the inlet pressure of the carrier gas (helium) was 100 kPa. For gas-liquid chromatographic analysis, nonadecanoic acid (C19:0) was used as an internal standard.

Assays

The activity of tTG (EC 2.3.2.13) was measured in the liver according to the method of Piacentini et al.[6] ME (EC 1.1.1.40) activity was assayed according to the method of Wise and Ball,[28] and TAT (EC 2.6.1.5) activity according to the method of Granner and Tomkins.[29] Protein levels were measured according to the method of Bradford[30] using a Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany). The serum cholesterol level was measured using a kit from Boehringer Mannheim (reference no. 237 574; Boehringer Mannheim, Meylan, France). Liver cholesterol was analyzed according to the method used by Starck and Madar[31] (extraction with hexane and determination using o-phthaldehyde[32]).

Statistical Procedure

Experimental values are expressed as the mean ± SEM. Results were compared using Student's *t* test. Differences between mean values were considered significant for *P* less than .05.

RESULTS

Serum and Hepatic Lipids

After 26 days of feeding a high-fat diet enriched with cholesterol and cholic acid, the body weight of the rats was not affected (352 ± 9 *v* 370 ± 10 g in control rats), although the liver weight was increased by 45% (19.29 ± 0.54 *v* 13.34 ± 0.64 g in control rats). In future studies with hypercaloric diets, it might be interesting to analyze the reason for this result, for instance, via study of body composition and/or activity levels. Rats fed the experimental diet had increased plasma and total liver cholesterol (Table 4). These rats also showed an increase in total FFAs in the liver and a modification of the relative portion of each series of FAs (less SFA and polyunsaturated FA [PUFA] and more monounsaturated FA [MUFA]).

Binding Properties of Receptors

The effects of the hypercholesterolemia-inducing diet on the maximum binding capacity and apparent affinity of nuclear receptors in the liver are reported in Fig 2 and Table 5. The cholesterol diet induced a decreased binding capacity for RAR, TR, and GR of 29%, 41%, and 35%, respectively, relative to the control value, but did not affect the apparent affinity of these receptors. The increase in liver mass (by 44% to 45%) in hypercholesterolemic rats induced a dilution of proteins, the concentration of which decreased 26% to 27% (cytosolic proteins, as well as proteins in nuclear extracts). However, the abundance of liver proteins was not significantly changed, so there was a decreased maximum binding capacity (expressed as femtomoles per milligram of nuclear proteins) in the liver (expressed as femtomoles per liver; total hepatic receptor number in hypercholesterolemic *v* control rats: TR, 3,184 *v* 5,372; RAR, 5,095 *v* 7,884; and GR, 15,466 *v* 21,783).

Table 4. Effects of a Hypercholesterolemia-Inducing Diet on Serum and Hepatic Lipids in Rats

Parameter	Control Diet	Hypercholesterolemia Diet
Serum cholesterol (mmol/L)†	2.65 ± 0.62	10.21 ± 3.50*
Liver cholesterol (μmol/g)†	23 ± 3	370 ± 53*
Total liver FFA (μg/g liver)‡	141.7 ± 8.5	251.3 ± 13.9*
% SFA	58	44
% MUFA	29	48
% PUFA	13	9

*Significantly different from control value using the Student *t* test ($P < .05$).

†Mean ± SEM from 10 rats.

‡Mean ± SEM from 2 to 5 different pools of 2 to 3 rats.

mRNA Levels

Results for quantitation of mRNAs coding for liver proteins that bind RA, T_3, or glucocorticoid hormones are shown in Table 6. In the liver of rats fed the hypercholesterolemia-inducing diet, the abundance of RNA for RAR, TR, and GR decreased approximately 20% to 30% relative to values in control rats. These decreased values were in agreement with the lower values for maximum binding capacity reported in these rats.

Enzymatic Activity

The activity of cytosolic enzymes whose expression is considered to be modulated by RA, T_3, or glucocorticoid hormones is reported in Table 7. These results were equivocal. Indeed, the activities of ME and TAT were decreased in rats fed a high-fat diet (in accordance with the results of the binding study of TR and GR), whereas the activity of tTG was markedly greater while the binding capacity of RAR was lower. These results provide evidence that in physiological conditions (rats fed experimental diets), the regulation of enzymatic activity is complex, involving more than just one hormone receptor and its effect on transcription.

DISCUSSION

A high-fat diet rich in saturated fat and cholesterol significantly reduced the expression of nuclear RAR, TR, and GR in rat liver. This experimental diet was hypercaloric and contained a high level of saturated fat and cholesterol. The high caloric level could account directly or indirectly, via changes in hormonal levels, for the observed results. But according to recent data, the nature of the dietary lipids must also be taken into account to explain the observed changes in cell signaling pathways.

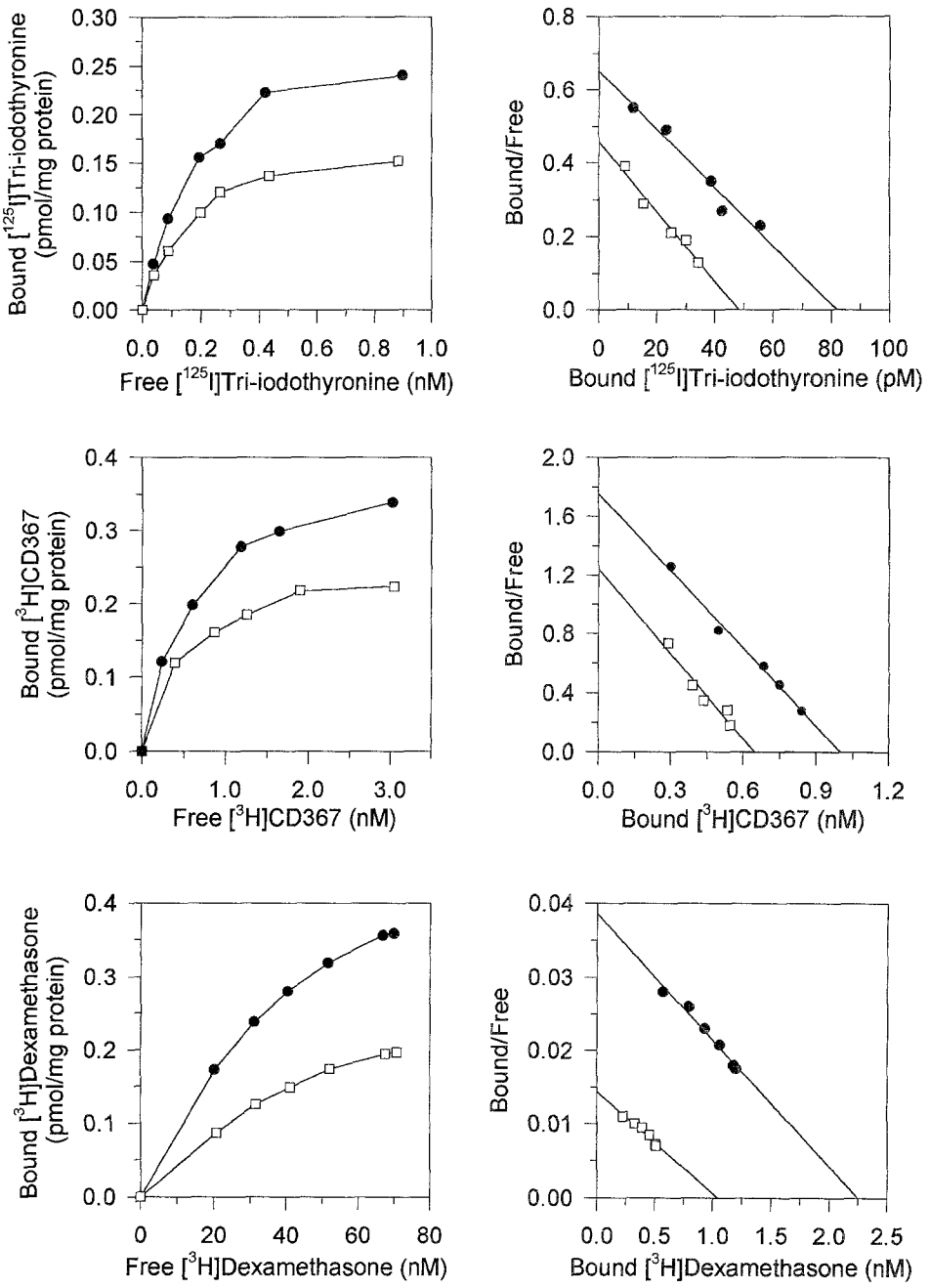

Fig 2. Examples of formation of nuclear receptor-ligand complexes. Left, formation of complexes at increasing concentrations of ligands; right, Scatchard analysis of ligand binding to nuclear proteins. Results are plotted as the ratio of bound to free ligand *v* bound ligand concentrations. Formation of complexes with [^{125}I]T_3 (top), [^{3}H]CD367 (middle), and [^{3}H]dexamethasone (bottom). Hepatic nuclear fractions were obtained from rats fed either a control diet (●) or a hypercholesterolemia-inducing diet (□).

Table 5. Effects of a Hypercholesterolemia-Inducing Diet on the Binding Properties of RAR, TR, and GR in Rat Liver

	C_{max} (mol/mg nuclear protein)		K_d ($\times 10^{-9}$ mol/L)	
Receptor	C Rats	H Rats	C Rats	H Rats
RAR	436 ± 29	310 ± 31*	0.65 ± 0.18	0.77 ± 0.16
TR	330 ± 46	194 ± 18*	0.13 ± 0.01	0.10 ± 0.01
GR	667 ± 52	432 ± 38*	55.0 ± 1.8	75.7 ± 8.6

NOTE. Data represent the mean ± SEM from 3 to 5 different pools of 2 to 3 rats (10 rats per diet; rats killed after 26 days of feeding).

Abbreviations: C, control diet; H, hypercholesterolemia-inducing diet (Table 1).

*Significantly different from control value using the Student *t* test ($P < .05$).

Table 6. Effects of a Hypercholesterolemia-Inducing Diet on mRNA Abundance of RAR, TR, and GR in Rat Liver (% β-actin mRNA)

Receptor	C Rats	H Rats
RARβ†	6.3 ± 0.1	4.8 ± 0.5*
TRα1,β1	5.1 ± 0.2	3.7 ± 0.5*
GR	15.6 ± 0.9	12.4 ± 0.7*

NOTE. Data represent the mean ± SEM from 4 to 5 different pools of 2 to 3 animals (10 rats per diet; animals killed after 26 days of feeding).

Abbreviations: C, control diet; H, hypercholesterolemia-inducing diet (Table 1).

*Significantly different from control value using the Student *t* test ($P < .05$).

†Isoform of RAR mainly expressed in liver.

‡Isoforms of TR that bind T_3.

Table 7. Effects of a Hypercholesterolemia-Inducing Diet on the Activity of tTG, ME, and TAT in Rat Liver

Parameter	C Rats	H Rats
tTG (pmol/h/mg protein)†	2.34 ± 0.05	4.04 ± 0.20*
ME (μmol/h/mg protein)‡	1.51 ± 0.04	0.41 ± 0.01*
TAT (μmol/h/mg protein)§	0.92 ± 0.01	0.76 ± 0.01*

NOTE. Data represent the mean value ± SEM from 3 to 5 different pools of 2 to 3 animals (10 rats per diet; animals killed after 26 days of feeding).

Abbreviations: C, control diet; H, hypercholesterolemia-inducing diet (Table 1).

*Significantly different from control value using the Student *t* test ($P < .05$).

†pmol of [^{3}H]putrescine incorporated into *N,N'*-dimethylcasein.

‡μmol of formed NADPH.

§μmol of formed *p*-hydroxyphenolpyruvate.

Some recent studies have shown the involvement of cholesterol in gene expression.[33] When cellular sterol levels are low, the expression of genes involved in cholesterol biosynthesis (hydroxymethyl glutaryl coenzyme A [HMG-CoA] reductase and HMG-CoA synthase) and uptake (low-density lipoprotein [LDL] receptor) is activated. Conversely, when sufficient cholesterol is present, the biosynthesis of these pivotal proteins is repressed. In the promoter of these genes, a motif termed the sterol regulatory element-1, or SRE-1, has been identified as the binding site for a common factor whose activity is modulated by sterols. It has also been shown in vivo that LDL receptor activity is downregulated by a cholesterol-containing diet high in palmitic acid or lauric and myristic acids.[34] Recently, Kawabe et al,[35] using cultured cells, demonstrated that FA synthase (FAS) mRNA expression is regulated by cholesterol. Thus, there are numerous experimental arguments in favor of a modulation of gene expression by the level of cholesterol, but to date, there is no information available concerning a direct effect of cholesterol on the expression of genes coding for nuclear receptors of the superfamily that contains RAR, TR, and GR.

Moreover, dietary FAs regulate gene expression.[36] This modulation of gene transcription could be the result of at least two distinct (but nonexclusive) mechanisms. Firstly, FAs may induce a modification of the cellular level of ligands, which in turn induces a modification of receptor expression. Since retinol esterification can restrict retinol for RA production, factors that influence the deposition of endogenous retinyl esters in cells are regulators of the cellular concentration of active retinoids. Some years ago, it was demonstrated in various cell types, including liver cells,[37] that exogenous unesterified FAs increase the esterification of retinol. In a recent study with cultured human epidermal keratinocytes, Randolph and Simon[38] showed that unsaturated FAs (particularly 16:1, 18:1, and 18:2) and SFAs (particularly with >14 carbons) reduced the rate of retinyl ester utilization and the cellular concentration of retinol and RA. Thus, these investigators claim that FAs have the potential to play a physiological role in modulating retinoid signaling by regulating cellular concentrations of active retinoids. Such a mechanism is interesting because in the rat liver the subtype RARβ is abundant[23] and upregulated by RA,[15,24] and thus a lower RA cellular content could be related to hypoexpression of RAR.

A similar mechanism could be taken into account to explain the lower expression of TR. Indeed, serum T_3 was lower in animals fed a saturated fat diet than in those fed a polyunsaturated fat diet.[39] Actually, serum T_3 was lower in our hypercholesterolemic rats (unpublished data, A. Martinez et al, 1996). Thus, since TR expression is upregulated by the T_3 level in the liver,[40] lower T_3 availability could be responsible for the hypoexpression of TR. Moreover, a lower level of T_3 could also help to explain the decreased expression of RAR, since in hypothyroid rats RAR expression is decreased in the liver.[25,41]

A similar hypothesis cannot be proposed for GR expression. Indeed, there is no available information on the expression of nuclear GR according to lipid status. It is only known that in the liver of immature rats the increased cytosol level of nonesterified FAs, resulting from heparin-induced lipolysis, decreases the binding capacity of cytosolic GR.[42] Moreover, a decreased level of the glucocorticoid hormone could not be related to a decreased expression of GR, in that GR expression in the liver is downregulated by glucocorticoid hormone.[43]

This discussion concerns a possible effect of FAs on the level of ligands such as RA or T_3, but an alternative hypothesis is that FAs interact with the cell signaling pathway by modifying the balance between nuclear receptors and thus avoiding the upregulation of TR and RAR. Indeed, these receptors bind to response elements of target genes as dimeric forms whose constitution includes not only RAR and TR but also RXR and peroxisome proliferator–activated receptors (PPARs). PPARs have been identified by Isseman and Green[44] as receptors that are activated by peroxisome proliferators. PPARs belong to the same superfamily of nuclear receptors that includes TR, RAR, RXR, and GR and constitute functional heterodimers with RXR.[45-48] Moreover, some arguments lead to the idea that FAs can activate PPARs[47,49] and PPARs, activated by FAs or products of their metabolism, are factors involved in the transcription of genes for lipolytic enzymes in rat liver.[47] In vitro studies have shown that TR, RAR, and PPAR compete to heterodimerize with RXR, so the effect of ligands on the gene transcription is influenced not only by the nature of the response element and the presence or absence of each ligand but also by the relative amount of each receptor. For instance, PPARs mediate cross-talk with TRs by competition for RXRs, and thus are able to selectively inhibit the transcriptional activity of TRs. These results were obtained with cultured cells[46] and with transgenic mice.[50]

In summary, evidence is provided herein that a diet characterized by a high caloric level of cholesterol and saturated fat induces decreased expression of nuclear TR, RAR, and GR in rat liver. These results could be due to numerous mechanisms, and studies on the relative role of each characteristic of the diet (calories, cholesterol, and saturated fat) are currently in progress. Whatever the mechanism(s) involved, these results highlight the role of dietary lipids in the modulation of the basic cell signaling pathways, in addition to their role as an energy source, essential membrane component, and eicosanoid precursor.

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