Biophysical Chemistry, 43 (1992) 255–263
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01665

Molecular motions and thermotropic phase behavior of triacylglycerols and cholesteryl esters in herpesvirus-infected arterial smooth muscle cells: A deuterium nuclear magnetic resonance study

David P. Hajjar [a], Francis Picart [b] and Kenneth B. Pomerantz [c]

[a] *Departments of Biochemistry and Pathology, Cornell University Medical College, New York, NY 10021 (USA)*
[b] *Department of Medicine, The Rockefeller University, New York, NY 10021 (USA)*
[c] *Department of Medicine, Cornell University Medical College, New York, NY 10021 (USA)*

(Received 28 August 1991; accepted in revised form 12 February 1992)

Abstract

The physical state of lipids in arterial smooth muscle cells (SMC) may contribute to lipid accumulation following injury. We have previously demonstrated that herpes simplex virus (HSV) infection alters the physical state of the neutral lipid accumulating in arterial SMC, as determined by differential scanning calorimetry (Biochem. J. 268 (1990) 693–697). To more precisely determine the molecular packing of neutral lipids in HSV-infected cells, the influence of HSV-infection on the thermotropic and phase-behavior of the lipids in intact arterial smooth muscle cells and in cell-free lipid extracts was evaluated using [^{2}H]-NMR, employing U-[^{2}H]-oleic acid incorporated into cells. Inspection of the [^{2}H]-line-widths indicate that the lipid of HSV-infected cells exhibited more restricted motion or a greater chemical shift dispersity than lipids from uninfected cells, as evidenced by significant broadening of the –CD=CD– signals at 25°C and 45°C. Fatty acid compositional analysis of the neutral lipids of control and HSV-infected cells following C18 : 1 supplementation (an amount added similar to the NMR experiments) reveals that: (1) there is approximately 55-fold more triacylglycerols (TG) than cholesteryl esters (CE) in control cells and 40-fold more TG than CE in the HSV-infected cells; (2) HSV infection significantly increases the C18 : 1 content of CE, and C18 : 3 and C20 : 4 in TG; and (3) HSV-infection does not alter the ratio of TG to CE. These data support the hypothesis that the greater restriction of the neutral lipids in HSV-infected cells may be due to the rigidifying effects of C18 : 1 on lipid mobility. Thus, alterations in the physical state of neutral lipids in HSV-infected cells may lead to reduced CE hydrolysis which, in turn, may contribute to or exacerbate lipid accumulation.

Keywords: Cholesteryl esters, Triacylglycerols, Deuterium NMR, Herpesvirus infection, Lipid physical state, Smooth muscle cells

1. Introduction

Cholesteryl esters (CE)* and triacylglycerols (TG) are the major neutral lipid constituents in vascular and non-vascular tissue, and represent storage pools of cholesterol and fatty acid, re-

Correspondence to: Dr. David P. Hajjar, Departments of Biochemistry and Pathology, Cornell University Medical College, 1300 York Ave., New York, NY 10021 (USA).

spectively [1,2]. Free cholesterol functions to maintain specific lipid/protein interactions and control membrane structural integrity [3]. These functions are dependent upon cholesterol trafficking from lysosomal and cytoplasmic pools to other membranes. Cellular TG is an important energy source in cells utilizing aerobic respiration, and is important in the maintenance of ATP generation via aerobic respiration. Although the TG pool is normally low, the flux of fatty acids through the TG pool is fairly high, suggesting its importance as a readily accessible source of fatty acid.

However, TG and CE often accumulate under specific pathological conditions, including cell injury, lipid storage diseases, and atherosclerosis [1]. The biochemical mechanisms responsible for neutral lipid accumulation are complex, and are due to specific enzyme deficiencies, defects in metabolism, hypoxia, alterations in exogenous (dietary) sources [1,2], and viral infection [4,5]. In studies previously reported by our laboratory, neutral lipid accumulates in human and non-human arterial smooth muscle cells (SMC) following herpesvirus infection. This is due principally to a reduction of CE hydrolases as a consequence of decreased DNA transcription and host protein synthesis [5–7] resulting in CE accumulation [6,7].

In additional experiments, we explored the possibility that herpesvirus infection of arterial SMC alters the physiochemical state of the esterified lipid, rendering it less susceptible to lipolysis [8]. Accordingly, the structural characteristics of the neutral lipid of SMC following HSV infection were examined by differential scanning calorimetry (DSC) [8]. Broadening of the transition temperature and a large increase in the absorbed enthalpy were observed by DSC measurements of HSV-infected cells relative to uninfected controls. These preliminary studies indicated that the neutral lipid was in a restricted state. Correlation of the DSC data with biochemical analysis of the neutral lipids in HSV-infected cells suggested that the increase in enthalpy was due to an increase in CE relative to TG, with a corresponding decrease in the ratio of polyunsaturated to saturated fatty acids. The physical state of neutral lipid does not favor hydrolysis, and may thus promote net neutral lipid retention [8].

Owing to the limitations of the DSC technique, no further predictions could be made regarding the conformation of the TG or CE in virally injured cells. Under favorable conditions, deuterium nuclear magnetic resonance ([^{2}H]-NMR) studies of labeled lipids can provide important details about the conformations and molecular packing of the hydrocarbon chains of CE and TG [9]. In addition, quadrupolar interactions provide a simple measure of the local order of the C–D bond. Thus, the use of [^{2}H]-NMR could provide additional information regarding the physical state of neutral lipids under normal conditions and under conditions of herpesviral infection. Accordingly, we report [^{2}H]-NMR spectra that characterize the molecular motions and thermotropic phase behavior of TG and CE accumulations following HSV infection of arterial SMC. These NMR studies are correlated with the TG and CE fatty acid composition; and, the CE/TG conformation in HSV-infected cells is proposed regarding the physiochemical nature of the lipid and its impact on the CE metabolic cycle.

2. Materials and methods

2.1 Materials

Medium 199, penicillin/streptomycin, and fungizone (amphotericin B) were obtained from Gibco (Grand Island, NY). Fetal-bovine serum (heat-inactivated) was purchased from Hyclone (Logan, UT). Bovine serum albumin (fatty-acid-free, fraction V) was purchased from Sigma Chemical Co. (St. Louis, MO). Perdeuterated U-[^{2}H]-oleic acid was purchased from Cambridge

Abbreviations used in this text: CE–cholesteryl ester, TG–triacylglycerol, C16:0–palmitic acid, C18:0–stearic acid, C18:1–oleic acid, C18:2–linoleic acid, C18:3–linolenic acid C20:4–arachidonic acid, SMC–smooth muscle cells, HSV–herpes simplex virus type 1, BSA–bovine serum albumin, PUFA–polyunsaturated fatty acids, SFA–saturated fatty acids, ACEH–acid cholesteryl ester hydrolase.

Isotope Laboratories (Woburn, MA). Triolein, diolein, monoolein, cholesteryl oleate, cholesterol methyl ester, and fatty acid methyl esters (C14:0 through C20:4, GLC standards) were obtained from Nu-Chek Prep (Elysian, MN). Diheineicosanoylphosphatidylcholine was obtained from Avanti Polar Lipids, (Pelham, AL). Boron trifluoride (15% in methanol) was obtained from Alltech (Deerfield, IL). All organic solvents (HPLC grade) and other biochemicals (reagent grade) were obtained from Fischer Scientific (Springfield, NJ).

2.2 Isolation and culture of rat aortic smooth-muscle cells (SMC)

Smooth muscle cells from thoracic aorta were isolated and cultured as previously described [6,7]. Cells were identified as arterial SMC by their 'hill and valley' configuration at confluence, as assessed by phase-contrast microscopy. Smooth muscle cells were induced to accumulate lipid by infection with HSV (HSV Type 1, from ATCC, Rockville, MD), as previously described [6,7]. Prior to analysis, cells were washed three times in phosphate-buffered saline (PBS, pH 7.4) containing 0.1% BSA, followed by washing in protein-free PBS.

2.3 NMR analysis

2.3.1 Preparation of intact cells

Confluent SMC (120×10^6) were mock-infected or infected with HSV (Type 1) as previously described [6] for 24 hours at a multiplicity of infection (MOI) of 0.05 in Medium 199 containing 10% serum and 40 mg U-[^{2}H]-oleic acid. Cells were then harvested into t 0% methanol in PBS after washing, and analyzed by NMR as described below. We stirred the cell preparation prior to NMR analysis since we observed some cell settling.

2.3.2 Preparation of cell lipid extracts

Confluent SMC (120×10^6) were mock-infected or infected with HSV (type 1) as previously described [6] for 24 hours at a multiplicity of infection (MOI) of 0.05 in Medium 199 plus 10% serum containing 40 mg [^{2}H]-oleic acid (deuterated in all positions). Cells were then harvested by scraping into PBS, centrifuged, and then lipid-extracted according to the method of Folch et al. [10]. The extracted lipid was then resuspended in PBS/ethanol for comparative NMR analyses to the intact cells [11].

2.3.3 NMR analysis

Deuterium NMR spectra were obtained on a wide-bore superconducting Nicolet Technologies NT-300 spectrometer operating at 300 MHz proton and 46 MHz deuterium frequencies running unlocked in the pulsed FT mode using quadrature detection. Spectra were generated from a standard one-pulse sequence and collected with 8192 real data points over a sweep width of -1 to 10 ppm, 507.098 Hz. The acquisition time was 4.04 s and an interpulse delay of 1 s was used. Sixty-four scans were acquired per data block and a 90° pulse of about 35 μs was used. The deuterium chemical shift scale was assigned approximately and indirectly by setting the D_2O offset of a standard sample of 100% D_2O to 4.67 ppm at the spectrometer frequency used to collect the spectra. Prior to Fourier transformation, the raw NMR data was treated with an exponential weighting function using a 1.0 Hz line broadening.

2.4 Neutral lipid analysis

Control and HSV-infected SMC (MOI of 0.5 for 2.0 hours) in 75 cm^2 flasks were incubated in M-199 containing 10% fetal bovine serum in the presence or absence of oleic acid (50 μg/ml) for 24 hours. After washing in PBS, cell lipids were extracted into hexane/propane-2-ol (3:2, v/v) according to the method of Hara and Randin [12]. Cell lipids were then separated on silica-gel H (Analtech) by sequential development in petroleum ether:ether:acetic acid (97:100:3, v/v/v) followed by petroleum ether:ether (97:3, v/v) [13]. The TG and CE regions were visualized under UV light using Rhodamine 6G. After application of 1,2-diheineicosanoylphosphatidylcholine (25 μg) to each spot, lipids were extracted into benzene, and derivatized to their corre-

sponding fatty acid methyl esters using 14% boron trifluoride in methanol. The corresponding fatty acid methyl esters were separated and quantified by GLC using a Hewlett Packard GLC (Model 5880), equipped with a 6 ft SP-2340 packed column (Supelco) employing a thermal gradient as follows: 150°C/4 min, 5°C/min to a final temperature of 215°C. Quantification of each fatty acid methyl ester was based on absorbance/mass ratios of methylheineicosanoate towards the absorbance areas of each unknown. In previous control studies, extraction of stock HSV-type 1 demonstrated no detectable TG or CE [6], indicating that the HSV preparation itself does not contribute neutral lipid to the cellular preparations.

All data were then normalized to protein, determined by the method of Lowry et al. using bovine serum albumin as standard [14].

2.5 Statistical analyses

Results of these experiments were analyzed either by Student's t test or by analysis of variance. Differences between treatments were evaluated using Fischer's test.

3. Results and discussion

In an attempt to define the mechanisms by which cholesterol crystals appear in atherosclerotic lesions, original studies by Small et al. revealed that complex mixtures of neutral and polar lipid exhibited limiting degrees of solubility, and precipitated cholesterol under conditions observed in atherosclerotic plaques [15]. Based on these observations, Small, and subsequently others, have demonstrated that the chemical compo-

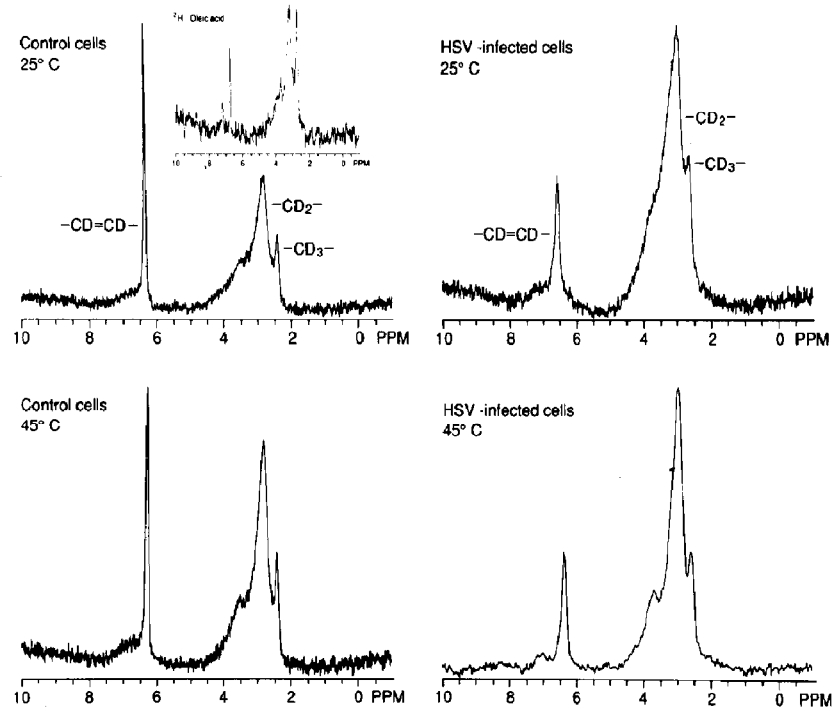

Fig. 1. Deuterium NMR spectra of uninfected (control) and herpesvirus (HSV)-infected arterial SMC following 24 h pulse of [^{2}H]-oleic acid at 25°C and 45°C. Cells were suspended in 4 ml PBS/ethanol for the NMR analyses. Insert: NMR spectra of the substrate alone, [^{2}H]-oleic acid, provided for reference purposes. The spectra shown is representative of three separate experiments. Line-width measurements for the control cells at 25°C are 2.5 Hz for the -CD=CD- group and 22.8 Hz for the $-CD_2-$ group; at 45°C, the -CD=CD- group linewidth is 5.1 Hz and the $-CD_2-$ group is 17.8 Hz. In the HSV-infected cells at 25°C, the -CD=CD- line-width is 7.6 Hz and the $-CD_2-$ is 33.0 Hz at 45°C, the -CD=CD- group line-width is 7.6 Hz and the $-CD_2-$ group is 17.8 Hz.

sition of cellular lipid can strongly determine its physical state and hence, its mobilization by cellular lipases [8,16,17]. Thus, alterations in the physical state of neutral lipid secondary to changes in its chemical composition may result from decreased TG/CE hydrolysis, leading to subsequent lipid accumulation.

We have previously demonstrated that HSV-infection of SMC results in significant CE accumulation, due, in part, to reduction in cholesteryl ester hydrolase activities [5–7,18]. However, the reduction in the lipase content was not sufficient to account for the degree of CE accretion. In additional studies, we then tested the hypothesis that the physical state of the neutral lipids that accumulate following HSV-infection may be such that they are poorer substrates for lipolytic enzymes, thus promoting net neutral lipid accumulation. Using differential scanning calorimetry, we discovered that the neutral lipids in sub-acute HSV-infected SMC were isotropic, due to a relative increase in CE relative to TG, with a concomitant shift toward saturated fatty acids [8]. However, due to the limitations of this technique, no further predictions could be made regarding the molecular packing of CE and TG, and the influence of alterations in the fatty acid composition to the phase behavior described by these lipids. To examine these questions, we employed [^{2}H]-NMR using uniformly labelled perdeuterated oleic acid as a lipid-specific probe to analyze order-disorder transitions and phase behavior of cellular lipid [9,11] in acutely infected cells, with preferential emphasis on TG and CE. As shown in Fig. 1, HSV infection of intact cells caused a marked broadening of the –CD=CD–NMR-signals (7.6 Hz) when compared to uninfected cells (2.5 Hz) at 25°C (at 45°C, the differences were 7.6 Hz vs 5.1 Hz). These findings suggest that the –CD=CD– bond in the environment of packed TG/CE molecules is either more constrained than in uninfected cells and/or it exhibits greater chemical heterogeneity. To determine if this effect was due specifically to the effects of HSV on alterations in cellular lipid, [^{2}H]-NMR spectra of cell lipid extracts following HSV-infection was also performed. The lipid extract contained mainly TG and CE which represents > 95% of

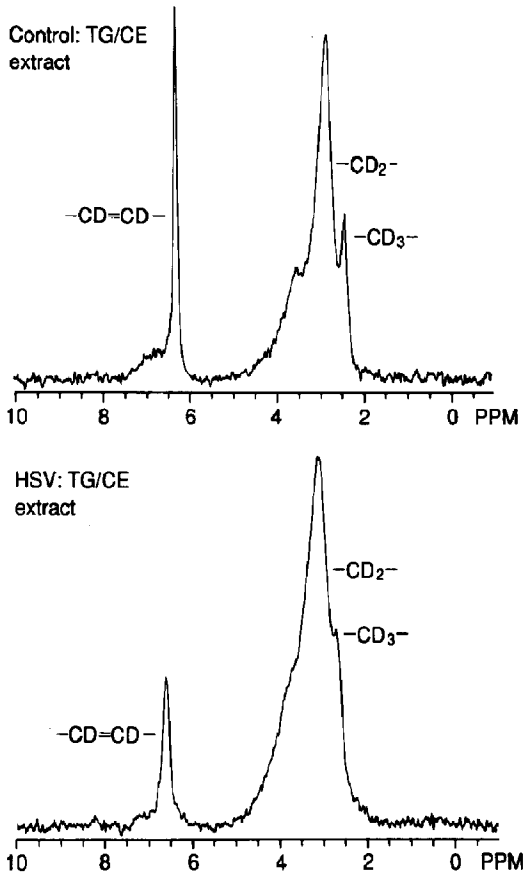

Fig. 2. Deuterium NMR spectra of cellular lipid extracts (composed mostly of TG and CE) from uninfected and HSV-infected arterial SMC at 25°C. Cells were exposed to 40 mg [^{2}H]-oleic acid/120×10^6 cells at 37°C for 24 h prior to harvest and lipid extraction. Extracts were resuspended in 4 ml ethanol/PBS for NMR analyses. The spectra shown in representative of two separate experiments. Using the control extract, line-width measurements for the –CD=CD– group is 5.1 Hz and 20.3 Hz for the –CD_2– group. Using the HSV extract, line-width measurements for the –CD=CD– group is 7.6 Hz and for the –CD_2– group, 35.5 Hz.

the oleic acid in free and esterified form. A large increase in the line width of the –CD=CD– NMR signals groups in the cell lipid extract derived from HSV-infected cells (7.6 Hz) compared to the cell lipid extract derived from control cells (5.1 Hz) was not only preserved, but also more apparent than in intact cells (Fig. 2). It appears that the broadening of the peaks are due to the

differences in the distribution of the oleic acid in the TG or CE, or to a greater rigidity of esterified lipid in the HSV-infected cells.

The biochemical elements which confer a particular physical state (anisotropy or restriction vs. isotropy) to neutral lipids include the relative amounts of TG and CE, as well as the fatty acid profile of each [8]. Since mono- and diacylglycerols constitute less than 5% of total neutral lipid, their contribution to the total neutral lipid compartment is of minor significance, and was not assessed in this study. Accordingly, the fatty acid mass and profile of TG and CE in uninfected and HSV-infected cells was determined. In

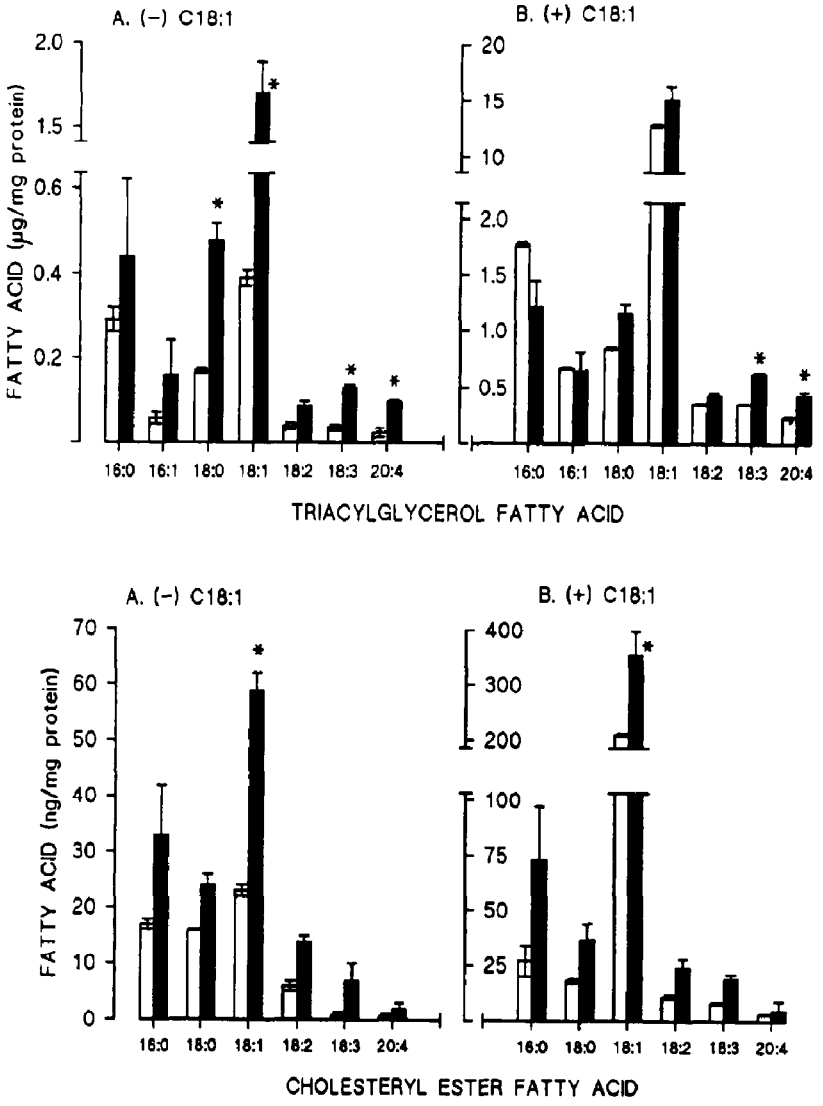

Fig. 3. Arterial SMC triacylglycerol (TG) and cholesteryl ester (CE) fatty acid composition: influence of HSV-infection and C18:1 supplementation. TG and CE from confluent cultures of control (open bars) and HSV-infected (closed bars) SMC incubated in the absence (panel A) and presence (panel B) of oleic acid were extracted into hexane/propan-2-ol (3/2, v/v), separated from other cell lipids by TLC, and transmethylated using BF_3/methanol (15%) and diheineicosanoylphosphatidyl choline (25 μg) as internal standard. The corresponding fatty acid methyl esters were quantified by GLC as described in Section 2.4. Results are expressed as mean ± standard error of measurement, each sample performed in duplicate.

addition, to evaluate the effects of the deuterated probe on potential alterations in lipid composition, uninfected and HSV-infected cells were exposed to exogenous (unlabelled) oleic acid prior to cell lipid analysis.

In Fig. 3(A), we show that HSV-infection of rat arterial SMC increased TG fatty acid content approximately 3-fold, from 1.0 ± 0.1 μg fatty acid/mg protein in the control cells to 3.1 ± 0.7 μg/mg protein following HSV infection. The alterations in the TG fatty acid profile in response to HSV infection are fatty acid specific; the largest increases are seen in C18:1 and in C18:0 on an absolute basis. The polyunsaturated fatty acids, C18:3 and C20:4, of TG are low in normal cells, but are also significantly increased by HSV infection (Fig. 3A). In Fig. 3(B), we show that oleic acid supplementation of uninfected SMC results in a 16-fold increase in total TG fatty acid (16.5 ± 0.2 μg/mg protein) compared to the results of the TG fatty acid profile shown in Fig. 3(A). Lipid analysis following C18:1 supplementation was done to assess the fatty acid profile of the TG (and CE) under conditions similar to the NMR experiments. Supplementation of HSV-infected SMC with C18:1 also leads to an increase in total fatty acid TG content (19.7 ± 2.5 μg/mg protein) compared to the results shown in Fig. 3(A) for the HSV-infected group. However, only C18:3 and C20:4 in the TG are increased relative to uninfected cells following C18:1 challenge.

A similar pattern of results were observed in the CE of arterial cells. There was little CE fatty acid in control cells (66 ± 2 ng/mg protein), most of which was C18:1, with lesser amounts of C16:0, C18:0, and polyunsaturated fatty acids (Fig. 3 A). HSV-infection of rat arterial SMC increased CE fatty acid approximately 2-fold (142 ± 26 ng/mg protein), which was due principally to an increase in C18:1 and C16:0 (Fig. 3A). Oleic acid supplementation of uninfected cells increased total CE fatty acid 4-fold (280 ± 20 ng/mg protein), due strictly to increased C18:1, with no significant alteration in the content of other fatty acids (Fig. 3B). Importantly, supplementation of HSV-infected SMC with C18:1 (Fig. 3B) increased total CE fatty acid content (514 ± 125 ng/mg protein).

Finally, to define more precisely which neutral lipid may be of quantitative importance in determining phase behavior, and to evaluate the possi-

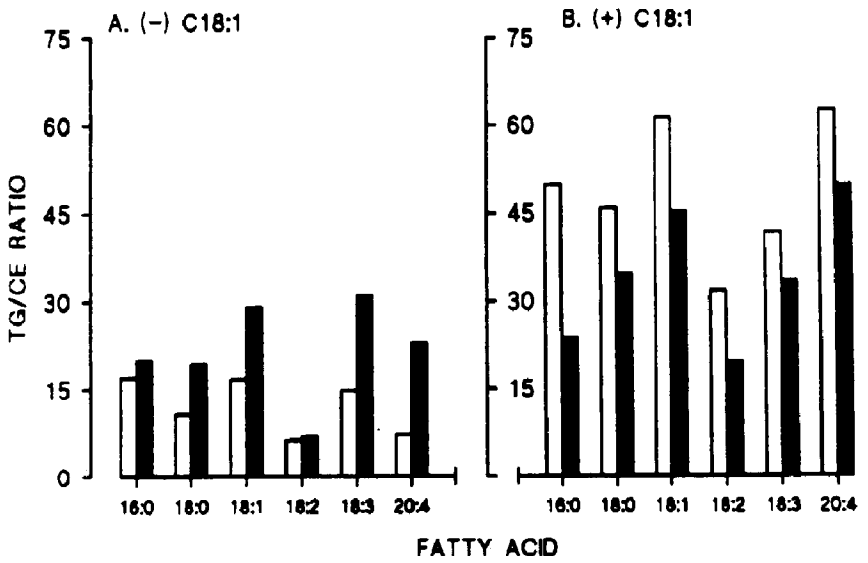

Fig. 4. HSV-infection increases the TG/CE ratio in arterial SMC (closed bars) in the absence of oleic acid (C18:1) supplementation (panel A) but not in the presence of oleic acid supplementation (panel B). Uninfected cells are represented by open bars. Results are expressed as mean of each ratio, each sample performed in duplicate.

bility that the trafficking of oleic acid into neutral lipid is preferential, the influence of HSV-infection and C18:1 supplementation on the TG/CE mass ratio was then examined. Control cells possess 12 ± 2 fold more TG than CE. The ratio of TG/CE in non-supplemented cells is summarized in Fig. 4(A). HSV-infection of SMC increased the TG/CE ratio to 23 ± 4, with specific increases in C18:1, C18:3, and C20:4 (Fig. 4A), indicating that HSV-infection preferentially increases the TG content. Oleic acid supplementation of control cells increased the TG/CE ratio over four-fold to 49 ± 5 owing to an increase in each fatty acid (Fig. 4B). This demonstrates that oleic acid, when added exogenously, is preferentially incorporated into TG, and may stimulate the incorporation of all other fatty acids into TG (Fig. 4B). Importantly, oleic acid supplementation of HSV-infected cells reduces the TG/CE ratio (34 ± 5) relative to uninfected cells co-incubated with exogenous oleic acid. There are consistent trends with each fatty acid (Fig. 4B), suggesting that oleic acid supplementation of HSV infection predisposes to increased CE accretion relative to TG.

Since the physical state of neutral lipid is also dependent upon the relative contribution of PUFA to SFA, the ratios of these fatty acids were compared. HSV-infection did not alter the PUFA/SFA ratio in either TG or CE relative to uninfected SMC (data not shown). However, oleic acid supplementation itself increased TG (0.5 ± 0.04 vs. 0.2 ± 0.04) and CE (0.5 ± 0.05 vs. 0.2 ± 0.05) PUFA/SFA ratio in uninfected cells, but did not alter the PUFA/SFA ratio in HSV-infected SMC (data not shown). These data demonstrate that while oleic acid itself can alter the PUFA/SFA ratio, the influence of this alteration may be minor since the major effect appears to be the oleic acid accumulation following HSV-infection.

In summary, the data described above demonstrate that HSV-infection of SMC stimulates TG and CE accumulation, with TG being the preferred lipid pool. The largest specific increase was in the C18:1 content, with lesser increments in C18:3 and C20:4. In addition, C18:1 increased the total fatty acid content of TG and CE, with TG the preferred pool; and, C18:1 supplementation reduced the specific effects of HSV infection on preferential C18:1 accumulation. These observations suggest that the increased broadening of the –CD=CD– spectra representative of the neutral lipid in HSV-infected cells relative to uninfected SMC was due principally to increased C18:1 in TG and CE.

There is a larger broadening of the –CD=CD– spectra in the HSV-infected cells as compared to the controls, particularly when C18:1 supplementation of both groups of cells produced no significant differences in the C18:1 content of TG in the cell (Fig. 3B). Although differences do exist in the C18:1 CE content between the HSV-infected cells and the controls, the CE contribution to the broadening of the line widths is less than the TG contribution since there are 40 times more TG than CE in the infected cells following C18:1 supplementation. We speculate that the altered CE may influence in some manner the physical state of TG in the HSV-infected cells which could contribute to that broadening of the –CD=CD– NMR peak. Or, it may be that the fatty acyl chains are arranged differently in the TG of the infected cells resulting in greater chemical heterogeneity and/or rigidity of the lipid which could explain the line-width broadening in these infected cells (Fig. 1).

Clearance of CE is believed to depend on its physical state, which is ultimately dependent upon the composition of the lipid droplet. In some studies, it has been demonstrated that anisotropic CE-rich inclusions present in Fu5AH and rabbit SMC efflux more slowly from cells than isotropic inclusions [19–21]. However, more recent work using model dispersions has revealed that there was no appreciable difference in the hydrolysis rates of lipid inclusions between isotropic and anisotropic inclusions [22]. Such differences could be ascribed to the interfacial properties and size of the lipid droplets, the latter a reflection of differences in their biochemical composition. Using model dispersions, the rate of hydrolysis of cholesteryl oleate by acid cholesteryl ester hydrolase (ACEH) was less than hydrolysis rates of lipid droplets containing cholesteryl linoleate or cholesteryl stearate. Furthermore, the rate of

cholesteryl oleate hydrolysis by ACEH was reduced in the presence of triolein [22], demonstrating that TG can effectively behave as an alternate substrate for this enzyme. These observations suggest that increased oleic acid may affect the interfacial packing of these lipids, rendering these lipids less accessible to CE hydrolases in the HSV-infected cells. We speculate that any rigidifying effects of oleic acid in the packing of TG and CE may prevent CE/ACEH association.

In human atherosclerotic lesions, non-esterified and esterified cholesterol exists in several physical states [17,23]. Fatty streaks are characterized by a single phase of CE in either an isotropic-liquid state or an anisotropic (disordered) smectic (liquid-crystal) state [24]. Advanced gruel plaques, which have a necrotic lipid-rich core, are characterized by three phases: (1) a cholesterol monohydrate crystal, (2) an oily CE phase, or (3) a lamellar phospholipid liquid-crystalline phase which is saturated with non-esterified and esterified cholesterol [17,23]. In either case, SMC accumulate CE/TG inclusions and evolve eventually into "foam" cells. Our findings now provide stronger evidence that injury to the vascular wall due to viral infection can lead to a predominance of CE and TG due not only to decreased translation of ACEH mRNA transcripts [7], but also due to the generation of neutral lipid droplets in the ordered, smectic, liquid-crystalline state which are hydrolyzed more slowly than CE in disordered, liquid state. These alterations may thus predispose to, or exacerbate lipid accumulation in response to injury.

Acknowledgements

The authors wish to thank Ms. Barbara D. Summers for expert technical assistance, and to Drs. David Cowburn and Esther Breslow for their critical assessment of the manuscript. This work was supported by grants from the National Institutes of Health (NIH), HL-18828, HL-45343 and HL-46403 awarded to DPH. NMR facilities at the Rockefeller University were purchased with grant support from the National Science Foundation, NIH, and the Keck Foundation.

References

1 E. Smith, Adv. Lipid Res. 12 (1974) 1.
2 R. Ross, N. Engl. J. Med. 314 (1986) 488.
3 M. Brown and J. Goldstein, Science 232 (1986) 34.
4 E. Benditt, T. Barrett and J. McDougall, Proc. Natl. Acad. Sci. USA 80 (1983) 6386.
5 D. Hajjar, J. Biol. Chem. 261 (1986) 7611.
6 D. Hajjar, K. Pomerantz, D. Falcone, B. Weksler and A. Grant, J. Clin. Invest. 80 (1987) 1317.
7 D. Hajjar, A. Nicholson, K. Hajjar, G. Sando and B. Summers, Proc. Natl. Acad. Sci. USA 86 (1989) 3366.
8 D. Hajjar, K. Pomerantz and J. Snow, Biochem. J. 268 (1990) 693.
9 Y. Parmar, H. Gorrissen, S. Wasasll and R. Cushley. J. Biol. Chem. 258 (1983) 2000.
10 J. Folch, M. Lees and G. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
11 J. Pearlman, J. Zajicek, M. Merickel, C. Carman, C. Ayers, J. Brookeman and M. Brown, Magn. Reson. Med. 7 (1988) 262.
12 A. Hara and N. Randin, Anal. Biochem. 90 (1978) 420.
13 D. Hajjar, T. Wight and S. Smith, Amer. J. Pathol. 100 (1980) 683.
14 O. Lowry, N. Rosebrogh, A. Farr and J. Randall, J. Biol. Chem. 193 (1951) 265.
15 D. Small and G. Shipley, Science 185 (1974) 222.
16 S. Katz, G. Shipley and D. Small, J. Clin. Invest. 58 (1976) 200.
17 D. Small, N. Engl. J. Med. 297 (1977) 873.
18 D. Hajjar, D. Falcone, C. Fabricant and J. Fabricant, J. Biol. Chem. 260 (1985) 6124.
19 L. Minor, G. Rothblat and J. Glick, J. Lipid. Res. 30 (1989) 189.
20 J. Glick, S. Adelman, M. Phillips and D. Rothblat, J. Biol. Chem. 258 (1983) 13425.
21 S. Adelman, J. Glick, M. Phillips and G. Rothblat, J. Biol. Chem. 259 (1984) 13844.
22 B. Lundberg, G. Rothblat, J. Glick and M. Phillips, Biochim. Biophys. Acta 1042 (1990) 301.
23 D. Small, Arteriosclerosis 8 (1988) 103.
24 B. Lundberg, Atherosclerosis 56 (1985) 93.