韓國營養學會誌 27(9) : 910~923, 1994
Korean J Nutrition 27(9) : 910~923, 1994

Effect of Individual Fatty Acids on Synthesis and Secretion of Apolipoprotein and Lipoprotein in Hep-G2 Cells*

Ryowon Choue** · Byung Hee Simon Cho

*Department of Food and Nutrition,** Kyung Hee University, Seoul, Korea*
Department of Food Science, University of Illinois at Urbana-Champaign, U.S.A

ABSTRACT

The effects of individual fatty acids, differing in their degree of unsaturation(18 : 0, 18 : 1, 18 : 2 and 18 : 3) on the biosynthesis and secretion of apolipoprotein and lipids were investigated in Hep-G2 cells. Synthesis of apolipoprotein was measured by the incorporation of ^{3}H-leucine into apolipoprotein(d<1.21g/ml) and synthesis of lipids was measured by the incorporation of ^{3}H-glycerol and ^{14}C-acetate into various lipid classes. Inclusion of 1.0mM of each fatty acids into the culture medium significantly increased the synthesis of total apolipoprotein and Apo B($p<0.05$). However, addition of fatty acid did not affect the synthesis of cellular and medium protein. Among different fatty acids tested, oleic acid had the greatest effect on Apo B synthesis. While stearic, linoleic and linolenic acid, all had similar effects. The secretion of triglyceride into the medium markedly increased in all fatty acid groups being 5−6 times over the albumin control. The triglyceride secretion was the highest in the oleic acid group. The secretion of phospholipid and cholesterol also increased with triglyceride output. A positive relationship existed between the output of lipoprotein-triglyceride and Apo B. Since the synthesis of Apo B was significantly increased when various fatty acids were included into the culture medium, part of the apparently stimulated synthesis of the apolipoprotein may be in response to the increased formation and secretion of lipoprotein lipids.

KEY WORDS : fatty acids · apolipoprotein · ^{3}H-leucine · lipoprotein · Hep-G2 cell.

Introduction

The major biosynthetic sites of lipoprotein are the liver and intestine[1]. Hepatic synthesis and secretion of lipoprotein are complex process which proceeds through several major biosynthetic pathways that require the synthesis of lipids (triglyceride, phospholipid and cholesterol) and apolipoprotein constituents[2-4]. Because of the essential role of apolipoprotein in the formation of the nascent very low density lipoprotein (VLDL) particles, the synthesis and secretion of the VLDL apolipoproteins are coordinately regulated with VLDL lipids[5][6]. The obligatory role of apolipoprotein for the lipoprotein secretion is

Accepted : October 17, 1993
*본 연구과제는 경희대학교 기초과학 연구소로부터 연구비 일부를 지원받았음.

recognized by the experiments in which an inhibition of protein synthesis limits the rate of VLDL secretion[7)8)]

The intracellular concentration of newly esterified triglyceride as well as other lipid metabolites is an important determinant of apolipoprotein release and possibly synthesis[9)]. Moreover, the chemical structure of triglyceride fatty acids that are derived from saturated or unsaturated fatty acids may modulate the secretion of apolipoprotein[10)] Then, we can speculate that individual fatty acids could regulate apolipoprotein synthesis differently. In contrast to the well established effects of fatty acids on the secretory rates of lipids, the role of fatty acids in the regulation of apolipoprotein secretion is controversial[11-14)]

The secretion of lipoprotein may vary quantitatively depending on the amount and types of cellular lipid synthesized in the liver[15)16)]. However, it has not been determined if alterations in the rate of hepatic lipid secretion are accompanied by parallel alterations in the release of apolipoproteins. Furthermore, it is not clear if individual fatty acids have a differential effect on apolipoprotein concentration. The purpose of the present study is 1) to study the differential effects of individual fatty acids on the synthesis and secretion of apolipoprotein and lipoprotein lipids, 2) to examine the newly synthesized apolipoprotein components under a basal culture condition, using Hep-G2 cell line.

Materials and Methods

1. Cell Cultures

The established Hep-G2 cell line, derived from human liver tumor cells, was obtained from American Type Culture Collection(ATCC, Rockvill, ML). The cells were grown in Dulbecco's Modified Eagle's Medium(DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin(100 units/ml) and streptomycin(100μg/ml). Stock cultures were maintained in T-25 flask at 37℃ under a humidified atmosphere of 95% air and 5% CO_2. The culture medium was changed every third day.

When cells were grown to 70−80% confluency, fresh cultures were initiated as described below. The growth medium was removed and the cells were washed twice with Ca^{2+}−, Mg^{2+}-free Balanced Hank's Salt Solution. The cells were then trypsinized with trypsin(2.5g/L)−EDTA(1g/L) solution followed by incubation at 37℃. The cell, suspended with complete medium, were mixed vigorously to disperse and an aliquot of the suspension(2.2×10^6 cells/T-25 flask) was seeded into T-25 flasks.

The cells reached confluence on the sixth day after seeding. The cultures then maintained a similar number of cells, approximately 11.5×10^6 cells per T-25 culture flask, for 5 additional days. In all experiments, cells were used on the sixth or seventh day of culture by which time a dense monolayer was formed.

2. Preparation of Fatty Acid/Albumin Complexes

A fatty acid/albumin complex solution was prepared under aseptic condition. Twenty μmoles of a fatty acid sodium salt(18 : 0, 18 : 1, 18 : 2 and 18 : 3) and 5μmoles(300mg) of essentially fatty acid-free bovine serum albumin were dissolved in DMEM culture medium(pH 7.4). The molar ratio of fatty acid to albumin was kept at 4 to 1(1.0mM of fatty acid/0.25 mM of albumin). The fatty acid/albumin complex solution was optically clear and was prepared fresh just prior to the experiment.

3. Incorporation of ^{3}H-Leucine into Apolipoprotein

To each culture flask was added : 2.5ml of se-

rum-free, leucine-free DMEM containing 1.0 nM of individual fatty acid/albumin complexes, or 0.25mM of albumin alone as a control and 5μCi of L-4, 5-^{3}H-leucine(1.64μCi/mg cell protein). The cells were then incubated for 4h at 37℃. After 4h incubation, the cells were harvested by trypsinization. The incorporation of radioactive leucine into the cellular proteins was estimated after the precipitation of an aliquot of cell suspension with 10% (v/v) trichloroacetic acid in the presence of excess unlabeled leucine. The radioactivity was determined using Bio-Safe II scintillation counting cocktail(Res. Products International Corp.) by Liquid Scintillation Spectrometry(Beckman, LS, 2800).

4. Sodium Dodecyl Sulfate-Polyacryl Gel Electrophoresis(SDS-PAGE)

SDS-PAGE was performed using a vertical slab gel apparatus, utilizing the discontinuous system described by Laemmli[17]. For the separation of apolipoproteins, linear gradient gels(4−22%) were used. The apolipoproteins were identified by comparison of their apparent molecular weights with those of the protein standards that were electrophoresed under identical condition. After electrophoresis, the gels were stained for 4h in methanol/acetic acid/water(50 : 10 : 40, v/v/v) containing 0.125% Commassie brilliant blue R-250 and then were destained in a methanol-acetic acid solution. The gels were dried in a Biogelwrap gel drying system(Bio Design Inc., NY) and the bands containing apolipoproteins were excised. The radioactivity of each apolipoproteins was measured after mixing the samples with Bio-Safe II scintillation counting cocktail. Radioactivity was determined in a Beckman Scintillation Spectrophotometer with 65% efficiency.

5. Incorporation of ^{3}H-Glycerol into Triglyceride and Phospholipid

To each culture flask was added : 2.5ml of serum-free DMEM medium containing 1.0mM of various fatty acid/albumin complexes or 0.25mM of albumin alone as a control and ^{3}H-glycerol(0.2 nM, specific activity 0.32μCi/nM). The cell medium and sonicated cell suspension were extracted twice with 5 volumes of chloroform/methanol (2 : 1, v/v) according to the method of Folch et al[18] Extracted lipid materials were separated into classes on glass fiber paper impregnated with silicic acid(Gelman Instrument Co., Ann Arbor, MI), as described previously by Cho[19] using a solvent system of petroleum ether : diethyl : acetic acid(85 : 15 : 1, v/v/v). The radioactivity of each class of lipids was counted in a Beckman Scintillation Spectrophotometer using scintillation cocktail containing 0.5% PPO and 0.03% dimethyl POPOP.

6. Statistical Analysis

Statistics and data compilation were conducted using the Statistical Package for the Social Science(SPSS, 1984) program at the University of Illinois at Urbana-Champaign. The Student's t-test was performed to compare the means between each groups.

Resutls

1. Effect of Fatty Acids on the Synthesis of Cellular and Medium Protein

Inclusion of 18 : 0, 18 : 1, 18 : 2 and 18 : 3 into the medium significantly increased the incorporation of ^{3}H-leucine into the total medium apolipoproteins(lipoprotein fraction, d<1.21g/ml)(Table 1). Among tested fatty acids, oleic acid had the greatest effect on apolipoprotein secretion, while stearic, linoleic and linolenic acid, all had similar effect. The incorporation of ^{3}H-leucine into cellular and medium protein(d>1.21g/ml) was not

Table 1. Effect of individual fatty acids on the incorporation of ^{3}H-leucine into the cellular and medium proteins

	Incubation conditions				
	Control	18 : 0	18 : 1	18 : 2	18 : 3
	dpm×10^{-3}/mg protein/4 h				
^{3}H-leucine in cell prot.	170 ± 14.4	176 ± 15.2	178 ± 12.0	172 ± 14.6	170 ± 13.7
^{3}H-leucine in d>1.21g/ml	50 ± 5.0	52 ± 6.6	54 ± 5.3	52 ± 8.2	50 ± 5.9
^{3}H-leucine in d<1.21g/ml	1.8± 0.1	2.1± 0.2^a	2.4± 0.1^{ab}	2.1± 0.1^a	2.1± 0.2^a

Monolayer of Hep-G2 cells were incubated with ^{3}H-leucine in the presence or absence of 1.0mM of individual fatty acids and the incorporation of ^{3}H-leucine into the specified protein fractions was determined as described in Materials and Methods.

All determinations were performed on fractions of cells and medium and represent the mean± standard error(n=6).

Values bearing superscripts a and b within a column are significantly different at $p<0.05$.

Table 2. Effect of individual fatty acids on the incorporation of ^{3}H-leucine into the various apolipoproteins

	Incubation conditions				
	Control	18 : 0	18 : 1	18 : 2	18 : 3
	dpm/mg cell protein/4 h				
Apo B	931± 33	1048± 66^a	1187± 84^{ab}	1096± 73^a	1072± 62^a
Apo E	249± 17	258± 16	252± 26	248± 15	257± 27
Apo A-I	414± 43	405± 25	417± 33	424± 24	413± 29
Apo C	38± 2	39± 3	41± 3	38± 3	39± 4

Monolayer of Hep-G2 cells were incubated with ^{3}H-leucine in the presence or absence of 1.0mM of individual fatty acids and the incorporation of ^{3}H-leucine into various apolipoproteins was determined as described in Materials and Methods.

All determinations were performed on fractions of cells and medium and represent the mean± standard error(n=6).

Values bearing superscripts a and b within a column are significantly different at $p<0.05$.

affected by the inclusion of individual fatty acids in the culture medium. Furthermore, there were no significant differences among the individual fatty acids tested.

2. Effect of Fatty Acids on the Synthesis of Various Apolipoproteins

As shown in Table 2, apo B was the major apoliporotein secreted by the cells incubated in the basal medium. Apo B comprised 57% of the total apolipoproteins followed by apo A-I(25% of the total), apo E(15% of the total) and apo C(2% of the total). The proportion of apolipoproteins secreted by the cells incubated with individual fatty acids remained the same as for the control, with apo B being the greatest, followed in order by apo A-I, apo E and apo C.

The inclusion of 1.0mM of individual fatty acids in the medium significantly increased the ^{3}H-leucine incorporation into secreted apo B compared to the control($p<0.05$). Among the fatty acids tested, oleic acid incubation resulted in the

most active incorporation of ^{3}H-leucine into apo B. The other fatty acids also had a stimulatory effect on the ^{3}H-leucine incorporation into apo B but to an extent that was significantly less than for oleic acid. There was no significant change in the ^{3}H-leucine incorporation into apo C, E and A-I with any fatty acid.

3. Apolipoprotein Profiles of Hep-G2 Cells

The apoliporpotein profile of lipoproteins after 4h incubation with individual fatty acids was determined by SDS-PAGE and the results are shown in Fig. 1. The identification of the major apolipoproteins was based on protein standards electrophoresed in the same system. As shown in Fig. 1, the newly secreted VLDL contained apo B, C, E and A-I. The major apolipoprotein constituent of the VLDL particle was apo B. The apparent molecular weight of the apolipoprotein was unaffected by incubation with fatty acid, as evidenced by their migration on the gel.

4. Effect of Fatty Acids on the Synthesis and Secretion of Triglyceride

Table 3 and Fig. 2 show the effect of 18 : 0,

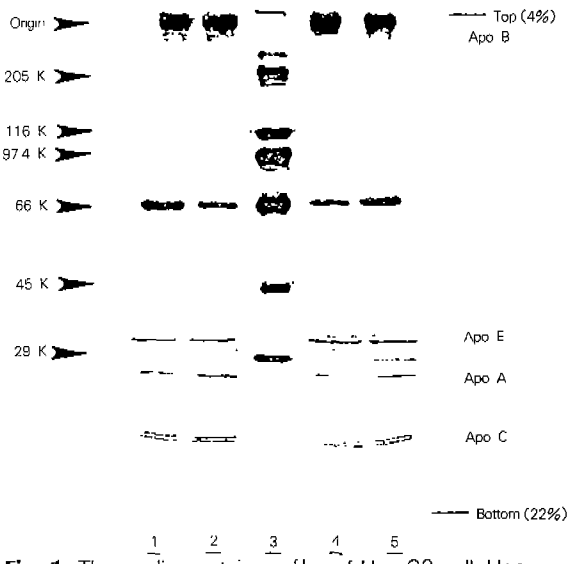

Fig. 1. The apolipoprotein profiles of Hep-G2 cell. Hep-G2 cells were incubated in serum-free, leucine-free medium containing 1.0mM of individual fatty acids and ^{3}H-leucine(lanes 1, 2, 4 and 5). The radiolabeled apolipoproteins were analyzed by SDS-PAGE. The samples were as follows ; lane 1, 2, 4 and 5 represent 18 : 0, 18 : 1, 18 : 2 and 18 : 3, respectively. The molecular weight markers(lane 3) are myosin(205 K), beta-galactosidase(116 K) phosphorylase(97.4 K), bovine albumin(66 K), egg albumin(45 K) and carbonic anhydrase(29 K).

Table 3. Effect of individual fatty acids on the incorporation of ^{3}H-glycerol into cellular and medium triglyceride

Incubation conditions	^{3}H-glycerol incorporation	
	Cellular TG[2)]	Medium TG
	dpm×10^{-3}/mg prot/4 h	dpm/mg prot/4 h
Control[1)]	19± 2	202± 34
18 : 0	213± 22[a]	944± 63[a]
18 : 1	205± 24[a]	1299± 172[ab]
18 : 2	175± 20[ab]	1156± 117[ab]
18 : 3	165± 23[ab]	993± 87[ab]

Hep-G2 cells were incubated for 4 h at 37℃ in 2.5ml of serum-free DMEM medium containing 1.0mM of individual fatty acids complexed to albumin(4 : 1) or albumin(0.25mM) alone as control and 0.2nM of ^{3}H-glycerol(S.A. 0.32μCi/nM). The incorporation of ^{3}H-glycerol into triglyceride was determined as described in Materials and Methods.

1) Values are mean± standard error of mean for three separate experiments, triplicate flasks of cells for each incubation condition.

2) Abbreviation : TG=Triglyceride

Values bearing superscripts a and b within a column are significantly different at $p<0.05$.

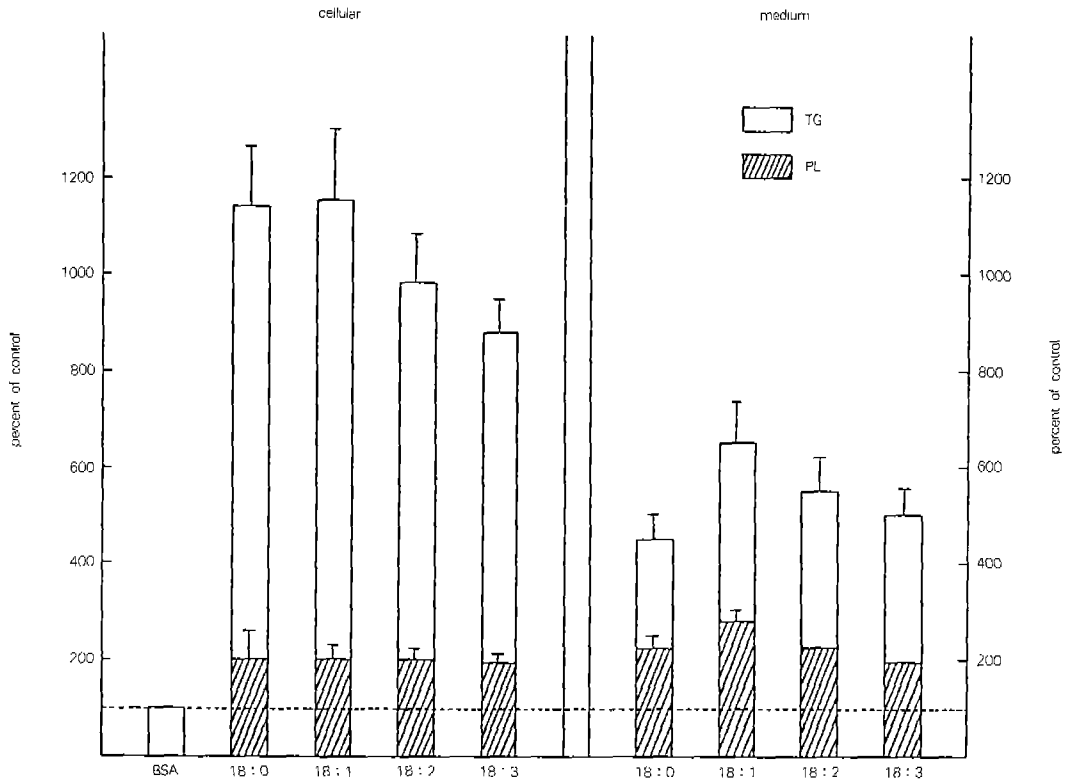

Fig. 2. Effect of individual fatty acids on the synthesis and secretion of triglyceride and phopholipid. This figure has been constituted from Table 3, 4

18 : 1, 18 : 2 and 18 : 3 on the cellular triglyceride synthesis and secretion as lipoprotein into the medium. In preliminary experiments, more than 95% of the ^{3}H-triglyceride secreted into the medium was isolated in a fraction having a density of less than 1,006g/ml. Therefore, the ^{3}H-triglyceride isolated from the medium was referred to as VLDL triglyceride secretion. The inclusion of 1.0mM of individual fatty acids stimulated both ^{3}H-triglyceride synthesis and secretion by 10- and 5.4-fold, respectively. Stearic and oleic acid had the greatest effect on the synthesis of cellular triglyceride. Linoleic and linolenic acid had a lesser effect on triglyceride synthesis compared to the stearic and oleic acid groups($p<0.05$).

The secretion of triglyceride was significantly higher for the unsaturated fatty acid group than for the saturated fatty acid. Oleic and linoleic acid had the highest effect on the stimulation of triglyceride secretion. Although the cellular triglyceride synthesis was the greatest with stearic acid, the triglyceride secretion into the medium was the lowest with this fatty acid.

5. Effect of Fatty Acids on the Synthesis and Secretion of phospholipid

In addition to the stimulation of ^{3}H-tryglyceride synthesis and secretion, inclusion of 1.0mM of fatty acid in the medium significantly increased ^{3}H-phospholipid synthesis by 2.1 fold($p<0.05$) (Table 4). The increased cellular phospholipid synthesis led to an increased lipoprotein phospholipid secretion into the medium by an average of 2.3-fold compared to the control. Among the

Table 4. Effect of individual fatty acids on the incorporation of ^{3}H-glycerol into cellular and medium phospholipid

Incubation conditions	^{3}H-glycerol incorporation	
	Cellular PL[2)]	Medium TG
	dpm×10^{-3}/mg prot/4 h	dpm/mg prot/4 h
Control[1)]	19.2± 1.7	124± 11
18 : 0	40.8± 5.8[a]	291± 24[a]
18 : 1	41.7± 3.9[a]	328± 26[a]
18 : 2	39.8± 3.3[a]	289± 19[a]
18 : 3	36.3± 3.0[a]	246± 23[ab]

Hep-G2 cells were incubated for 4 h at 37℃ in 2.5ml of serum-free DMEM medium containing 1.0mM of individual fatty acids complexed to albumin(4 : 1) or albumin(0.25mM) alone as control and 0.2nM of ^{3}H-glycerol(S.A. 0.32μCi/nM). The incorporation of ^{3}H-glycerol into phospholipids was determined as described in Materials and Methods.

1) Values are mean± standard error of mean for three separate experiments, triplicate flasks of cells for each incubation condition.

2) Abbreviation : TG=Phospholipid

Values bearing superscripts a and b within a column are significantly different at $p<0.05$.

Table 5. Effect of individual fatty acids on the incorporation of ^{14}C-acetate into cellular and medium cholesterol

Incubation conditions	^{14}C-acetate incorporation	
	Cellular chol.[2)]	Medium chol
	dpm×10^{-3}/mg prot/4 h	dpm/mg prot/4 h
Control[1)]	20.5± 2.7	409± 33
18 : 0	31.8± 4.7[a]	568± 68[a]
18 : 1	29.6± 0.7[a]	525± 68[a]
18 : 2	28.2± 2.3[a]	530± 44[a]
18 : 3	27.4± 1.0[ab]	476± 38[a]

Hep-G2 cells were incubated for 4 h at 37℃ in 2.5ml of serum-free DMEM containing 1.0mM fatty acid complexed to albumin(4 : 1) or albumin(0.25mM) alone as control and 0.1mM of ^{14}C-acetate(S.A. 2.5μCi/nM). The incorporation of ^{14}C-acetate into cholesterol was determined as described in Materials and Methods.

1) The data represent the mean± standard error of mean for three separate experiments, triplicate flasks of cells for each incubation.

2) Abbreviation : Chol.=Cholesterol

Values bearing superscripts a and b within a column are significantly different at $p<0.05$.

fatty acids tested, there were no significant differences in the incorporation of ^{3}H-glycerol into cellular phospholipid. The secretion of ^{3}H-phospholipid was similar under the influence of 18 : 0, 18 : 1 and 18 : 2, but was significantly lower with 18 : 3.

6. Effect of Fatty Acids on the Synthesis and Secretion of Cholesterol

Table 5 shows the effect of individual fatty acids on the incorporation of ^{14}C-acetate into cellular cholesterol and lipoprotein cholesterol secreted into the medium. Inclusion of individual fatty acids in the cell culture medium significantly stimulated the incorporation of ^{14}C-acetate into cellular and lipoprotein cholesterol compared to the control($p<0.05$). The incorporation of ^{14}C-ace-

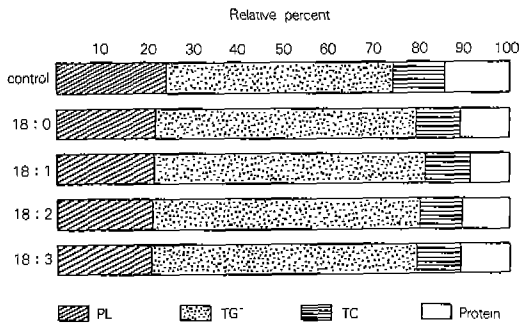

Fig. 3. Relative percentage of total cholesterol, triglyceride, phospholipid and protein of lipoproteins secreted by Hep-G2 cells after 4 h incubation with 1.0 mM of individual fatty acid/albumin complexes and albumin(0.25mM) alone for a control.

tate into cellular cholesterol was similar under the influence of 18 : 0, 18 : 1 and 18 : 2, but was significantly lower with 18 : 3. No significant difference was observed for ^{14}C-labeled lipoprotein cholesterol secretion into the medium among the different fatty acids tested.

The VLDL secreted by cells incubated with individual fatty acids has different ratio of apolipoprotein to each lipid classes(Fig. 3). Saturated fatty acid has a lower percentage of triglyceride and higher percentage of cholesterol and phospholipid.

Discussion

The human hepatoma cell line, Hep-G2 cells, has been shown to express many functions attributed to the normal human hepatocyte[4)5)14)]. The present study also demonstrates that Hep-G2 cells actively synthesize and secrete apolipoproteins (apo B, C, E and A-I) and lipoprotein lipids(TG, PL, C). Furthermore, the responsiveness of Hep-G2 cells to fatty acids documented in the present study provides further evidence for the usefulness of this cell line in studying the regulation of hepatic lipoprotein metabolism.

The inclusion of fatty acids stimulated the secretion of apolipoprotein, especially apo B. This elevated apo B secretion in the presence of fatty acids has also been observed in other studies using perfused rat liver and cultured rat hepatocyte[14)16)20)]. Fatty acids with a chain-length greater than 16 have been shown to be more effective in apo B synthesis in the rat liver[21)22)]. The results of this study found that all 18-carbon fatty acids tested, increased the incorporation of ^{3}H-leucine into apo B secreted into the medium.

Apo B was the major apolipoprotein in the culture medium, followed in order of decreasing concentration by, apo A-I, E and apo C. Thus the composition of apolipoproteins in the culture medium differed from that in plasma where apo A-I is the major apolipoprotein[23)]. In addition, there was a higher content of apo E in the culture medium(15%) when compared to the plasma(3.5 %)[24)]. These differences could be explained by the intestinal contribution of apolipoproteins A-I and A-II *in vivo* and a different residence time of apolipoproteins in the plasma[25)].

Apo C has been shown to be acquired by VLDL after hepatic secretion[26)]. This suggests an independence of apo C from VLDL formation and secretion. As shown in Table 2, the synthesis of apo C was not affected by the presence of fatty acids which increased VLDL secretion into the medium. The association of apo C with VLDL may be related to the size or lipid composition of the VLDL particle, this may then result in the reported modulation of VLDL catabolism by these apolipoproteins[27)].

The inclusion of fatty acids in the medium also stimulated the synthesis of triglyceride. The net increase in hepatocyte triglyceride synthesis was dependent on the fatty acid concentration in the medium(data is not shown). These results support the conclusion drawn from an earlier in vivo

study[28] that high serum free fatty acid levels can directly contribute to development of fatty liver. The stimulation of triglyceride synthesis by individual unsaturated fatty acids was closely correlated to the increased secretion of lipoprotein-triglyceride(Table 3). Examination of the data indicates an inverse relationship between the number of carbon-carbon double bonds and the ability to stimulate triglyceride synthesis or secretion of these three 18-carbon fatty acids tested. Similar findings have also been reported in rat hepatocytes[6] and perfused rat liver[13].

It is noteworthy that the triglyceride secretion into the medium was the lowest with stearic acid, although cellular triglyceride synthesis was the highest with this fatty acid. This lower rate of triglyceride secretion could be explained by the fact that the apparent Michaelis constant(Km) for the overall sequence of reactions leading to the secretion of triglyceride is larger for stearic acid than for oleic or linoleic acid[13]. It also could be explained by the fact that maximal rates of triglyceride secretion in the presence of stearic acid occurs only when the metabolic pool reaches an appropriate concentration. Reduced triglyceride secretion in the presence of stearic acid was also reported by Kohout et al[9].

In agreement with others[29-31], linolenic acid had a smaller effect on triglyceride synthesis and secretion compared to the other fatty acids tested. This suggests that a possible mechanism by which n3 polyunsaturates lower triglyceride level in plasma may be due to an inhibition of triglyceride synthesis in the liver, thereby reducing the triglyceride content in VLDL secretion.

The inclusion of fatty acids also stimulated phospholipid synthesis and secretion(Table 4). These findings, along with the previously discussed finding that fatty acids increase the synthesis of triglyceride, suggest that the secretion of triglyceride and phospholipid may be coupled. This may partly explain the development of fatty liver in response to choline deficiency or choline analogue supplementation[32-34]. If phospholipid required for the secretion of VLDL and is not available, then triglyceride will accumulate in the liver.

The addition of fatty acids to the culture medium had a significant effect on the synthesis and secretion of cholesterol(Table 5). The degree of stimulation was dependent on the amount of exogenous fatty acid available. Previous research[35][36] has shown that increased triglyceride secretion, induced by increased fatty acid availability, stimulated HMG-Co A reductase activity. It is known that the stimulating effect of excessive free fatty acid on cholesterol synthesis contributes to an increase in the size of the cholesterol ester storage pool in the liver[37]. The intracellular ratio of free to esterified cholesterol decreases in the presence of excess free fatty acids[5].

VLDL secreted by cells incubated with saturated fatty acid has a lower percentage of triglyceride and a higher percentage of cholesterol and phospholipid[5][38]. A similar effect was observed in this experiment(Fig. 3). It has been known that as the proportion of phospholipid and cholesterol relative to triglyceride increases there is a decrease in the size of the lipoprotein particles[12][39]. Smaller lipoprotein particles are known to be more dense[38]. These observation may have physiological consequences for the body. The more dense VLDL produced in the presence of saturated fatty acid are metabolized in the blood more slowly. This will result in a longer retention time for these particles and therefore, an elevated cholesterol level in the blood.

The current study demonstrates that the increased secretion of triglyceride induced by fatty acid is accompanied by an increased apolipoprotein secretion. This suggests that the increased availa-

bility of fatty acid to the liver may require an increased apo B secretion to transport the elevated lipids as VLDL particles. In contrast to the marked enhancement of triglyceride secretion to the medium(5.4-fold), the addition of individual fatty acids resulted in only small to moderate, but statistically significant increases in the concentration of apo B(1.2-fold). These results for triglyceride and apo B suggest that the induction of the synthesis for neutral lipids and apolipoproteins is not enhanced to the same degree. It appears that the fatty acid induced change in the composition of VLDL particles is a consequence of the association of apolipoprotein with larger, triglyceride-rich lipoproteins. These particles contain a higher ratio of triglyceride to apo B than the corresponding particles isolated from basal medium. When considered in conjunction with an increase in the triglyceride/apo B ratio in the medium, the present finding seems to suggest that in Hep-G2 cells the VLDL apolipoproteins are assembled into the secretory vesicles with variable amounts of lipid.

Conclusions

The liver is a mjor site for the synthesis of the plasma lipoproteins and plasma lipid concentration is strongly influenced by the quantity and composition of fats in the diet. However direct studies of the regulation of lipoprotein synthesis and secretion by human liver using individual fatty acids are lacking. On the basis of the present study, it appears that apolipoprotein release and possibly synthesis might be modulated by the intracellular concentration of newly esterified triglyceride and/or some other lipid metabolites which could be affected by the structure of fatty acids included in the medium. A positive relationship existed between the secretion of lipoprotein-triglyceride and Apo B. Therefore, part of the apparent stimulated synthesis of the apolipoprotein may be in response to the increased formation and secretion of lipoprotein lipid.

Literature cited

1) Wong L, Piano RM. Biogenesis of very-low density lipoproteins in rat liver : Intracellular distribution of apolipoprotein B. *Eur J Biochem* 164 : 357-367, 1987
2) Dolphin PJ. Lipoprotein metabolism and the role of apolipoproteins as metabolic programmers. *Can J Biochem* 63 : 850-869, 1985
3) Dashti K. McConathy WJ, Ontko JA. Production of apolipoproteins E and A-I by rat hepatocytes in primary culture. *Biochim Biophys Acta* 618 : 347-358, 1980
4) Dashti K, Wolfbaure G. Secretion of lipids, apolipoproteins and lipoproteins by human cell line, Hep-G2 : Effects of oleic acid and insulin. *J Lipid Res* 28 : 432-436, 1987
5) Ellsworth JL, Erickson SK, Cooper AD. Very low and low density lipoprotein synthesis and secretion by the human hepatoma cell line Hep-G2 : Effects of free fatty acid. *J Lipid Res* 27 : 858-874, 1986
6) Davis RA, Boogaerts JR. Intrahepatic assembly of very low density lipoproteins : Effects of fatty acids on triglycerol and apolipoprotein synthesis. *J Biol Chem* 257 : 10908-10913, 1982
7) Davis RA, Engelhorn SC, Weinstein DB, Steinberg D. Very low density lipoprotein secretion by cultured rat hepatocytes : Inhibition by albumin and other macromolecule. *J Biol Chem* 255 : 2039-20 45, 1980
8) Alcindor LG, Infante B, Soler-Argilaga C, Paisonnier A, Polonovske J, Caroli J. Induction of the hepatic synthesis of beta-lipoproteins by high concentration of fatty acids : Effects of actinomycin D. *Biochim Biophys Acta 210 : 483-486, 1970*
9) Kohout M, Kohoutova B, Heimberg M. The effects of chain length and degree of unsaturation of the fatty acid substrate on the output of triglyceride by the liver. *Biochim Biophys Acta* 210 : 177-179,

1970

10) Salam WH, Wilcox HG, Heimberg M. Stimulation by oleic acid of incorporation of L-[4, 5-^{3}H] leucine into very low density lipoprotein apoprotein by isolated perfused rat liver. *Biochem Biophys Commun* 132 : 28-34, 1985

11) Petersburg SJ, Madeley A, Robinson DS. A study of the interrelationship between the triacylglycerol and protein components of very-low-density lipoproteins using the perfused rat liver. *Biochem J* 150 : 315-321, 1975

12) Wilcox HG, Dunn GD, Heimberg M. Effects of several common long chain fatty acids on the properties and lipid composition of the very low density lipoprotein secreted by the perfused rat liver. *Biochim Biophys Acta* 398 : 39-54, 1975

13) Kohout M, Kohoutova B, Heimberg M. The regulation of hepatic triglyceride metabolism by free fatty acids. *J Biol Chem* 246 : 5067-5074, 1972

14) Rash JM, Rothblat GH, Sparks CE. Lipoprotein apolipoprotein synthesis by human hepatoma cells in culture. *Biochim Biophys Acta* 661 : 294-298, 1981

15) Heimberg M, Wilcox HG. The effect of palmitic acid and oleic acid on the properties and composition of the very low density lipoprotein secreted by the liver. *J Biol Chem* 247 : 875-880, 1972

16) Bell-Quint J, Forte T. Time related changes in the synthesis and secretion of very low density, low density and high density lipoproteins cultured rat hepatocytes. *Biochim Biophys Acta* 663 : 83-98, 1981

17) Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 227 : 680-685, 1970

18) Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from aniaml tissues. *J Biol Chem* 226 : 497-509, 1957

19) Cho BHS, Kummerow FA. Fatty acid incorporation into cholesteryl ester-and phospholipid by arterial subcellular fractions of swine. *Proc Soc Exp Biol Med* 155 : 8-12, 1977

20) Ruderman NB, Richards KC, Valles de Bourges V. Regulation of production and release of lipoprotein by the perfused rat liver. *J Lipid Res* 9 : 613-619, 1968

21) Petit D, Raisonnier A, Amit N, Infante R. Lack of induction of VLDL apoprotein synthesis by medium chain fatty acids in the isolated rat liver. *Ann Nutr Metab* 26 : 279-286, 1982

22) Patsch W, Tamai T, Schonfeld G. Effect of fatty acids on lipid and apoprotein secretion and association in hepatocyte cultures. *J Clin Invest* 72 : 371-378, 1983

23) Alaupovic P. Serum apolipoprotein profiles of dyslipoproteinemias. In : Dietary fats and health. Perkins EG, Visek WJ. pp574-597, American Oil Chemists' Society, Champaign, IL, 1983

24) Sparks CE, Hnatiuk O, Marsh JB. Very low density lipoprotein synthesis and secretion in HepG2 cells. *Can J Biochem* 57 : 786-790, 1982

25) Wu AL, Windmueller HG. Relative contribution by liver and intestine to individual plasma apolipoproteins in the rat. *J Bio Chem* 254 : 7316-7322, 1979

26) Windler E, Chao YS, Havel RJ. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins. *J Biol Chem* 255 : 8303-8307, 1980

27) Wilcox HG, Heimberg M. Secretion and uptake of nascent hepatic very low density lipoprotein by perfused livers from fed and fasted rats. *J Lipid Res* 28 : 351-360, 1987

28) Feigelson EB, Pfaff WW, Karmen A, Steingerg D. The role of plasma free fatty acids in development of fatty liver. *J Clin Invest* 40 : 2171-2180, 1961

29) Spady DK, Woollett LA. Interaction of dietary saturated and polyunsaturated triglycerides in regulating the processes that determine plasma low density lipoprotein concentrations in the rat. *J Lipid Res* 31 : 1809-1819, 1990

30) Grundy SM. Effects of fatty acids on lipoprotein metabolism in Man. In : Health effects of polyunsaturated fatty acids in seafoods. Simopoulos AP, Kifer RR, Martin RE. pp153-171, Academic Press

Inc, Orlando, 1986

31) Marsh JB, Topping DL, Nestel PF. Comparative effects of fish oil and carbohydrate on plasma lipids and hepatic activities of phosphatide phosphohydrolase, diacylglycerol acyltransferase and neutral lipase activities in the rats. *Biochim Biophys Acta* 922 : 239-243, 1987

32) Lombardi B, Ani P, Schlunk FF. Choline deficiency fatty liver : Impaired release of hepatic triglyceride. *J Lipid Res* 9 : 437-446, 1968

33) Kushwaha RP, Jensen LS. Effect of choline deficiency on utilization of labeled precursors of choline and turnover of choline in the liver of nature Japanese Quail. *J Nutr* 105 : 226-232, 1975

34) Pelech SL, Pritchard PH, Brindley DN, Vance DE. Fatty acids promote translocation of ATP : Phosphocholine dytidylyltransferase to the endoplsmic synthesis. *J Bio Chem* 258 : 6782-6788, 1983

35) Brown MS, Dana SE, Goldstein JL. Regulation of 3-hydroxy-3-methylglutary coenzyme A reductase activity in cultured human fibroblasts. *J Bio Chem* 249 : 789-796, 1974

36) Goh EH, Heimberg M. Effects of free fatty acids on activity of hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase and on secretion of triglyceride and cholesterol by liver. *J Biol Chem* 252 : 2822-2826, 1979

37) Goh EH, Heimberg M. Stimulation hepatic cholesterol biosynthesis by oleic acid. *Biochem Biophys Res Commun* 55 : 382-388, 1973

38) Wilcox HG, Dunn GD, Heimberg M. Effects of a mixture of a saturated with an unsaturated fatty acid on secretion of the very low denstiy lipoprotein by the liver. *Biochem Biophys Res Commun* 73 : 733-740, 1976

39) Heimberg M, Wilcox HG. The effects of palmitic and oleic acids on the properties and composition of the very low density lipoproteins secreted by the liver. *J Biol Chem* 247 : 875-880, 1972

Effect of Fatty Acids on Apolipoprotein Synthesis

= 국문초록 =

지방산이 Hep-G2 Cell Line에서 Apolipoprotein과 지질의 합성 및 분비에 미치는 영향

조 여 원**·조 병 희

경희대학교 식품영양학과**

Department of Food Science, University of Illinois at Urbana-Champaign, U.S.A

Stearic acid(18 : 0), oleic acid(18 : 1), linoleic acid(18 : 2), 그리고 linolenic acid(18 : 3)를 Hep-G2 cell 배양액에 첨가하여 아포지단백질(apolipoprotein)과 지단백질(lipoprotein)의 합성 및 분비에 이 지방산들이 미치는 영향을 조사하였다. ^{3}H-leucine을 사용하여 아포지단백질의 합성을 측정하였으며 ^{3}H-glycerol과 ^{14}C-acetate로 triglyceride, phospholipid 그리고 cholesterol의 합성을 측정하였다. 각각의 지방산 1.0mM을 배양액에 첨가하여 배양한 결과 total 아포지단백질과 apo B의 합성이 유의성있게 증가되었으며 세포내의 단백질이나 배양액 중의 단백질 합성에는 변화가 없었다. 실험한 지방산중 oleic acid가 apo B 합성에 가장 큰 영향을 미쳤으며 다른 지방산들간에는 유의성있는 차이가 나타나지 않았다($p>0.05$). Apo E, apo A-I, apo C의 합성은 지방산 첨가에의해 영향을 받지않았으며 지방산 종류에 따라서도 유의적인 영향을 받지 않았다. 또한 이들 지방산은 triglyceride, phospholipid, cholesterol의 합성과 분비에도 현저한 증가를 나타냈다($p<0.01$). 지방산 종류에 따라 지질 합성에 미친 영향은 유의적으로 다르게 나타났으며 실험한 지방산 중 oleic acid가 가장 큰 영향을 미침으로 apo B 합성에 미친 영향과 유사한 결과를 나타냈다. 특히 주목할 사항은 이들 지방산들 중 stearic acid는 중성지방의 합성에 가장 큰 영향을 미쳤으나 분비(secretion)에는 가장 적은 효과를 나타냄으로 합성과 분비에 상관관계를 나타낸 다른 지방산들과 상이한 결과를 보였다. 지질 분비와 Apolipoprotein 합성 사이에 서로 positive한 상관관계가 나타났으며 이는 지방산 첨가후 세포내에 과도로 축적된 triglycerdie를 VLDL의 형태로 분비하고자 apo B의 합성이 증가된 것으로 사려된다.

중심단어 : fatty acid · apolipoprotein · ^{3}H-leucine · lipoprotein · Hep-G2 cell.