

# SUPPRESSION OF VLDL-TRIACYLGLYCEROL SECRETION BY BOTH $\alpha$ - AND $\beta$ -ADRENOCEPTOR AGONISTS IN ISOLATED RAT HEPATOCYTES

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## ABSTRACT

The effects of alpha and beta-adrenergic stimulation on triacylglycerol secretion were investigated in isolated rat hepatocytes. Epinephrine within 3h of incubation suppressed triacylglycerol secretion by 35% and increased its cellular content by 18%. The inhibitory effect of epinephrine was abolished by inclusion of phentolamine and also prazosin but not with propranolol. Trifluoperazine concealed the inhibitory effect of epinephrine in a dose-dependent manner, whereas theobromine did not have any significant effect. The secretion of triacylglycerol was suppressed not only by the  $\alpha$ -agonist phenylephrine but also by the  $\beta$ -agonist isoproterenol. Dibutyryl-cyclic AMP also inhibited secretion of triacylglycerol by approximately 30%. The results indicate that epinephrine suppressed triacylglycerol secretion via the  $\alpha_1$ -adrenoceptor whereas stimulation of beta—as well as alpha—adrenoceptors can exert a similar effect. Calcium-calmodulin dependent protein kinase may be involved in the down-regulation of VLDL secretion. The unexpected effect of isoproterenol has been discussed in relation to “dual signaling” and also the “store-dependent calcium entry” hypotheses.

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## INTRODUCTION

Synthesis, assembly and secretion of VLDL associated components are subject to hormonal and metabolic regulations and recently have been reviewed.<sup>1-6</sup> Secretion of VLDL is not only suppressed by calcium-linked agents such as catecholamines,<sup>7-9</sup> prostaglandins,<sup>10</sup> and calcium antagonists,<sup>11,12</sup> but also by agents that act via the cAMP pathway including glucagon,<sup>13-15</sup> cAMP derivatives<sup>15-17</sup> and cAMP-dependent protein kinase.<sup>15</sup> Although secretion of VLDL is regulated by both signal transduction systems (i.e., calcium and cAMP pathways), epinephrine acts mainly through the  $\alpha_1$ -

adrenoceptor in the liver.<sup>18</sup>

Calcium may be involved in the secretion of VLDL through one or more of at least four processes: I) Synthesis of secretory products. Calcium-mobilizing agents regulate the activities of the enzymes involved in glycerolipid biosynthesis.<sup>19-21</sup> Alpha-adrenergic stimulation inhibits lipogenesis and leads to channeling of fatty acid substrate from esterification to oxidation pathway.<sup>22</sup> II) Post-transcriptional modifications and subsequent translocation through the secretory pathway. Intraluminal calcium is required for the actions of signal peptidase, chaperone and apoprotein-B (apo-B) itself.<sup>4</sup> The optimum concentration of  $Ca^{2+}$  within the endoplasmic reticulum (ER) may be essential for proper folding, assembly and secretion of nascent apo-B.<sup>4,23</sup> This is supported by the actions of prostaglandins and also verapamil that selectively inhibit VLDL secretion but have no influ-

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ence on any parameters of hepatic lipid metabolism.<sup>10</sup> III) Regulation of microtubules and microfilaments, and IV) Fusion of secretory vesicles to the plasma membrane. Secretion of VLDL is achieved via a general mechanism for protein secretion and is microtubule dependent, and is inhibited by colchicin.<sup>24</sup> The increment in cytosolic calcium in the liver cells in contrast to some other secretory cell types, has been accompanied with an inhibition of the secretory process.

In the present study, the effects of various agents effective to adrenoceptors have been investigated on triacylglycerol secretion in rat hepatocytes. The data indicates that epinephrine suppresses triacylglycerol secretion via the  $\alpha_1$ -adrenoceptor, whereas both  $\alpha$ - and  $\beta$ -agonists can lead to a similar effect.

## MATERIALS AND METHODS

### Chemicals

Dibutyryl adenosine 3',5'-cyclic monophosphate ( $Bt_2$ -cAMP), (-)epinephrine, oleic acid, bovine serum albumin (essentially fatty acid free) and trifluoperazine were obtained from Sigma (USA). Collagenase (5000 U/mg protein) was purchased from Merck (Germany), prazosin from Pfizer (USA) and theobromine from BDH (England). The sources of all other chemicals were as previously described.<sup>25,26,34</sup>

### Hepatocyte isolation

Hepatocytes were isolated from Spargue-Dawley rats weighing 250-300 g that had free access to laboratory chow and water.  $L_4$ -hepatocytes were isolated by two-step collagenase perfusion technique as described previously.<sup>26</sup> Trypan blue exclusion was greater than 90% and less than 10% of total LDH was released.

### Hepatocyte incubation

Hepatocytes ( $8 \pm 0.5$  mg protein/mL) were incubated for 3h at 37°C in a total volume of 4 mL of Krebs-Ringer bicarbonate (KRB) containing 0.5% (w/v) bovine serum albumin, 0.25 mM oleate, 20 mM glucose and 2.5 mM  $CaCl_2$  in siliconized flasks shaking at 90 cycles/min under an atmosphere of  $O_2:CO_2(19:1)$ .

### Lipid analyses

Incubation was stopped on ice and the medium removed after centrifugation at 1500 g for 3 min. The medium was centrifuged at 12000 g for 10 min at 4°C to remove broken cells and cell debris and the total volume of the supernatant was extracted for lipid analysis according to Folch et al.<sup>27</sup> Manipulative losses of triacylglycerol during extraction were accounted for by addition of glycerol [ $^{14}C$ ]-trioleate as internal standard. The recovery of glycerol [ $^{14}C$ ]-trioleate was determined by scintillation counting.<sup>14</sup> The cell pellet was washed twice by ice cold homogenization solution<sup>28</sup> and

sonicated by an ultrasonic processor and extracted for lipid analysis as described previously.<sup>26</sup> The mass of triacylglycerol was measured by using a kit purchased from Zist-Shimi, Iran (Triglycerides GPO-PAP). VLDL secretion was estimated by measuring the appearance of triacylglycerol in the incubation medium as it is well established that more than 95% of medium triacylglycerol is in the form of VLDL.<sup>28</sup> The dependence of secreted triacylglycerol to VLDL fraction was assessed by precipitation and ultracentrifugation methods as described by Mangiapane & Brindley.<sup>28</sup>

### Other analytical procedures

Protein concentration was determined by the method of Lowry et al.<sup>29</sup> LDH was assayed by a colorimetric method, using a diagnostic kit (Sigma).

### Statistical analysis

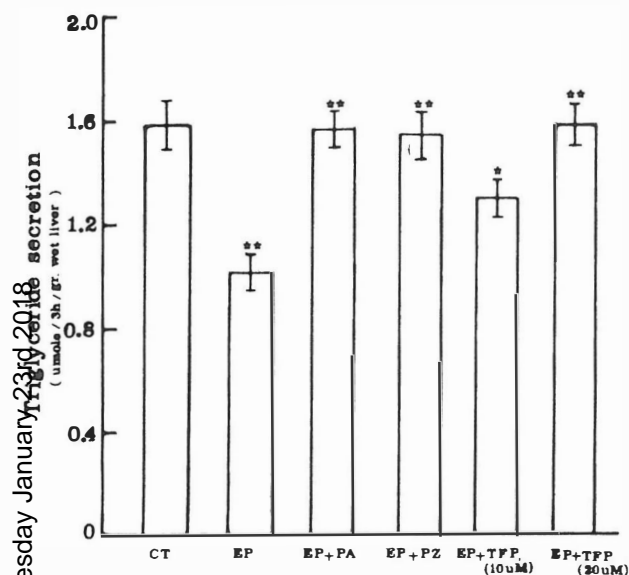
The results were expressed as mean  $\pm$  S.E.M. The statistical significance of any observed differences was tested by Student's t-test.

## RESULTS

The effect of epinephrine on triacylglycerol secretion in the absence and the presence of  $\alpha$ -adrenoceptor antagonists are shown in Fig. 1. The mass of triacylglycerol was measured in the lipid extract of the incubation medium. The rate of triacylglycerol secretion was  $1.58 \pm 0.09$  ( $\mu$ mole/3h/g wet liver) in the absence of epinephrine. Epinephrine at  $10 \mu M$  concentration suppressed triacylglycerol secretion by about 35% ( $p < 0.005$ ). The inhibitory effect of epinephrine was abolished in the presence of  $10 \mu M$  phentolamine, a general  $\alpha$ -antagonist. Trifluoperazine, an anticalmodulin drug, abolished the effect of epinephrine in a dose dependent manner by about 50% at  $10 \mu M$  and completely at  $20 \mu M$ .

The effect of epinephrine on triacylglycerol secretion in the absence and the presence of cAMP-linked agents is shown in Fig. 2. The values for triacylglycerol secretion for control and epinephrine-treated samples are as in Fig. 1. The inhibitory effect of epinephrine has remained unchanged in the presence of propranolol (PRO,  $10 \mu M$ ). In addition, theobromine (TB,  $1 \mu M$ ), a cAMP-phosphodiesterase inhibitor, did not have any significant effect on epinephrine action. Dibutyryl-cAMP ( $Bt_2$ -cAMP,  $0.1$  mM) also inhibited triacylglycerol secretion alone by approx. 30% ( $p < 0.01$ ).

The effect of epinephrine, phenylephrine and isoproterenol on triacylglycerol secretion are depicted in Fig. 3. The rate of triacylglycerol secretion in the presence of phenylephrine (PE,  $10 \mu M$ ) and isoproterenol (ISO,  $10 \mu M$ ) was  $1.17 \pm 0.07$  and  $1.23 \pm 0.09$  ( $\mu$  mole/3h/g wet liver), that implies 25% and 22% inhibition, respectively. None of the antagonists examined alone have any significant effect on triacylglycerol secretion (data not shown).

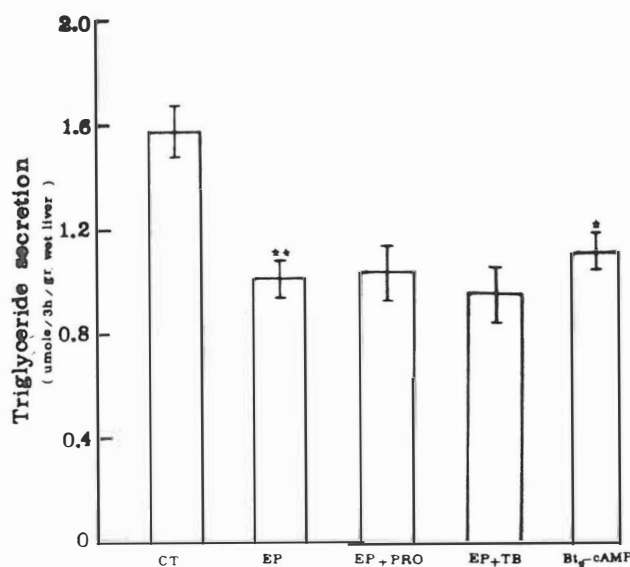


**Fig. 1.** Effects of epinephrine on triacylglycerol secretion in the absence and the presence of  $\alpha$ -antagonists. Hepatocytes at a concentration of  $8 \pm 0.5$  mg protein/mL were incubated 3h in KRB containing  $20 \mu\text{M}$ -glucose and  $0.25 \mu\text{M}$ -oleate in a total volume of 4mL at  $37^\circ\text{C}$ . Hepatocytes were preincubated for 5 min with  $10 \mu\text{M}$ -phenolamine (PA),  $1 \mu\text{M}$ -prazosin (PZ) and 10, 20  $\mu\text{M}$ -trifluoperazine (TFP). The rate of triacylglycerol secretion was expressed as  $\mu\text{mole TG}/3\text{h}/\text{g}$  wet liver. It is assumed that  $1 \mu\text{mole}$  of triacylglycerol is 885 mg and one gram wet weight of liver is equal to 158 mg total cell protein. Results are expressed as the mean  $\pm$  S.E.M. of four interassays performed at least in three different cell preparations. The epinephrine treated sample was compared to the control and the others to the epinephrine treated one. \* indicate  $p < 0.025$  and \*\* indicate  $p < 0.005$ , respectively.

The effect of epinephrine on cellular triacylglycerol content is shown in Fig. 4. The mass of triacylglycerol was measured in the lipid extract of the cells. Hepatocyte triacylglycerol levels were  $9.64 \pm 1.0$  and  $11.31 \pm 0.86$  ( $\mu\text{mole}/\text{g}$  wet weight of liver) in the absence and presence of epinephrine respectively, which implies about 18% increment ( $p < 0.05$ ). The stimulatory effect of epinephrine has been antagonized by prazosin but retained in the presence of propranolol. In every case, the total triacylglycerol contents of the system (the sum of the cell and incubation medium) was almost constant.

## DISCUSSION

The present results indicate that epinephrine decreases the secretion of triacylglycerol and increases its content in isolated rat hepatocytes. The observation that the inhibitory effect of epinephrine on triacylglycerol secretion was antagonized by a general  $\alpha$ -adrenoceptor antagonist (phenolamine) and also by a specific  $\alpha_1$ -antagonist (prazosin), but not with the  $\beta$ -antagonist propranolol suggests  $\alpha_1$ -receptor involvement. This idea was also supported by the influence



**Fig. 2.** Effects of epinephrine on triacylglycerol secretion in the absence and the presence of cAMP-linked agents. The values of triacylglycerol secretion of control and epinephrine treated samples are as in Fig. 1. Hepatocytes were preincubated for 5 min with  $10 \mu\text{M}$ -propranolol (PRO) and  $1 \text{mM}$ -theobromine (TB). All other experimental conditions are as described in the legend of Fig. 1. The epinephrine and dibutyryl-cAMP ( $\text{Bt}_2\text{-cAMP}$ ,  $0.1 \text{mM}$ ) treated samples are compared to control and the others to the epinephrine treated one. \*, \*\* indicate  $p < 0.01$  and  $p < 0.005$ , respectively.

of trifluoperazine (TFP)—but not theobromine—on the action of epinephrine.  $\alpha_1$ -Suppression of triacylglycerol secretion is in accordance with previous reports.<sup>7,9</sup> TFP is a competitive inhibitor of calmodulin and can attenuate signaling mediated by it.<sup>30,31</sup> Results presented here demonstrated that TFP concealed the inhibitory effect of epinephrine in a dose-dependent manner. Therefore, calcium-calmodulin multiprotein kinase (CaM-MPK) may be involved in the regulation of VLDL secretion. This is the first demonstration that activation of the calcium-calmodulin pathway is required for down-regulation of VLDL secretion. Microtubule associated protein-2 (MAP-2), Tau factor and probably tubulin itself have been shown to be phosphorylated by CaM-MPK.<sup>31</sup> Phosphorylation by CaM-MPK promotes the disassembly and inhibits the rate of reassembly of microtubules.<sup>31</sup> CaM-MPK also participates in  $\alpha$ -adrenergic stimulation of ketogenesis.<sup>22</sup> Alpha-1 stimulation of hepatocytes is associated with a release of calcium from ER to the cytosol.<sup>32</sup> Release of intracellular calcium was found to be accompanied by calcium influx across the plasma membrane into the cytosol by a mechanism known as “store-dependent calcium entry”.<sup>33</sup> Therefore,  $\alpha_1$ -stimulation leads to the simultaneous depletion of calcium stores of ER and an increment in the cytosolic calcium concentration, both of which may cause suppression of VLDL secretion. Thus far, more attention has been focused on the former and it has been deduced that an optimum concentration of  $\text{Ca}^{2+}$  within

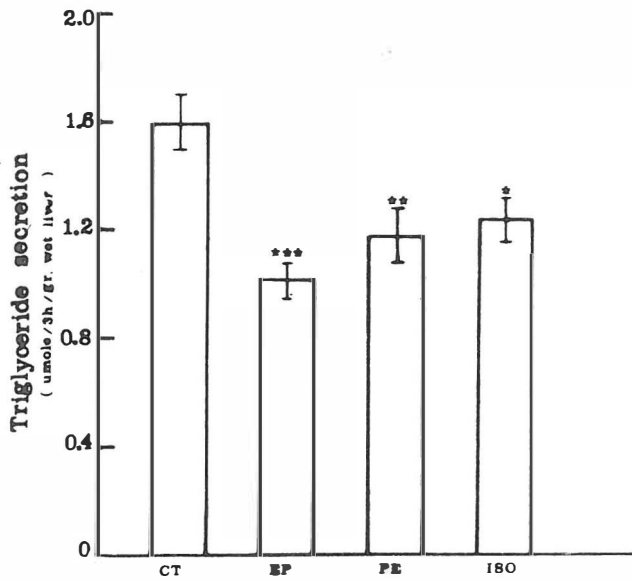


Fig. 3. Effects of epinephrine,  $\alpha$ - and  $\beta$ -adrenoceptor agonists on triacylglycerol secretion. The values of control and epinephrine treated samples are as in Fig. 1. Hepatocytes were incubated 3h with epinephrine (EP,  $10\mu\text{M}$ ), phenylephrine (PE,  $10\mu\text{M}$ ), and isoproterenol (ISO,  $10\mu\text{M}$ ). Results are shown as mean  $\pm$  S.E.M. for at least three different cell preparations. All samples are compared to control. \* $p < 0.025$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ .

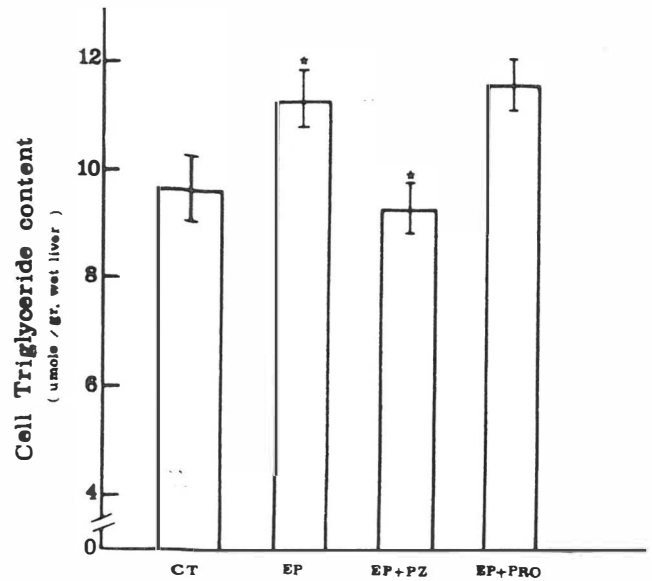


Fig. 4. Effects of epinephrine on cellular triacylglycerol content in the absence and presence of  $\alpha$ - and  $\beta$ -antagonists. Hepatocytes were incubated for 3h in KRB as described in the legend of Fig. 1. Hepatocytes were preincubated for 5 min by  $1\mu\text{M}$ -prazosin (PZ) and also  $10\mu\text{M}$ -propranolol (PRO). Results are expressed as the mean  $\pm$  S.E.M. of four interassays performed at least in three different cell preparations. The epinephrine treated sample was compared to the control and the others to the epinephrine treated one. \* $p < 0.05$ .

the ER may be essential for proper folding, translocation and secretion of apo-B.<sup>4</sup> But increasing cytosolic calcium via  $\text{Ca}^{2+}$ -calmodulin may also be involved in the inhibition of VLDL secretion. However, further investigation is required to elucidate the role of  $\text{Ca}^{2+}$ -calmodulin in the regulation of VLDL secretion.

The fact that the effect of epinephrine is mediated via the  $\alpha_1$ -receptor does not necessarily exclude the involvement of cAMP in the regulation of VLDL secretion. In fact hepatic VLDL metabolism is regulated by both signal transduction systems.<sup>7-17,34</sup> The present data also shows that  $\text{Bt}_2$ -cAMP diminishes triacylglycerol secretion. This agrees with results obtained in perfused rat liver,<sup>16</sup> hepatocytes in incubation<sup>17</sup> and in culture.<sup>15</sup> By now, both calmodulin- and cAMP-dependent protein kinases may participate in suppression of VLDL secretion.

The most notable observation of the present investigation was the significant inhibition of triacylglycerol secretion, not only by the  $\alpha$ -adrenoceptor agonist phenylephrine but also by the  $\beta$ -agonist isoproterenol. This is the first report for unexpected inhibitory effect of isoproterenol on VLDL secretion. A number of agents acting alone at a single receptor appear capable of generating more than one second messenger, i.e. dual signaling.<sup>35</sup> Glucagon<sup>36-37</sup> and isoproterenol<sup>33,37</sup> can raise both intracellular  $\text{Ca}^{2+}$  and cAMP in rat hepatocytes. Isoproterenol via activation of phospholipase-C (PLC) induces a rapid increase in inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) and consequently  $\text{Ca}^{2+}$ -mobilization.<sup>37</sup> It has

been proposed that the effect of isoproterenol is achieved by activation of the  $\beta$ -adrenoceptor and mediated via different G-proteins,  $G_q$  and  $G_s$ , that lead to activation of PLC and adenylate cyclase, respectively.<sup>35</sup> Regulation of VLDL secretion by isoproterenol and glucagon may be instances in which both  $\text{Ca}^{2+}$  and cAMP act together. Cross-talking is present within and between the two signal transduction pathways, at receptors, G-proteins, effectors and second messengers.<sup>35</sup> The cAMP analogues and forskolin, an adenylate cyclase activator, also raised cytosolic calcium in rat hepatocytes.<sup>37</sup> Some evidence demonstrated that this was achieved by direct activation of PLC via  $G_q$ ,<sup>37</sup> whereas interaction at further points is also probable.<sup>35</sup> Cyclic-AMP induces phosphorylation of the  $\text{IP}_3$ -receptor and modifies it more sensitive to  $\text{IP}_3$ .<sup>33</sup> At the present time, it seems that some of the effects of cAMP are exerted indirectly through affecting calcium homeostasis. However, whether the effects of activation of the cAMP pathway are exerted directly or at least partially indirectly via  $\text{Ca}^{2+}$  mobilization, and the points at which  $\text{Ca}^{2+}$  and cAMP interact to regulate VLDL secretion remains to be studied.

The results also indicate that epinephrine in the presence of propranolol increases the cellular triacylglycerol content significantly. This observation is in agreement with previous work<sup>26</sup> but nevertheless differs from the results reported by Brindle & Ontko.<sup>7</sup> The fact that, in the presence

of epinephrine, net accumulation of intracellular triacylglycerol was small, implies that it is exposed to intracellular degradation. In the previous work the time course of cellular lipids revealed that cellular triacylglycerol and phospholipid contents in the presence of epinephrine were higher than the control at all times, and the differences were constant along the time.<sup>26</sup> Since the differences were constant during the time course<sup>26</sup> and the total triacylglycerol content of the system (sum of the cell and incubation medium) were constant in the presence and absence of epinephrine, the accumulated lipids within the cells represent only those lipids that have not been secreted yet. Therefore, it seems that epinephrine inhibits triacylglycerol secretion via exertion of an inhibitory effect on the secretory pathway.

## REFERENCES

- Gibbons GF: Assembly and secretion of hepatic very low density lipoprotein. *Biochem J* 268: 1-13, 1990.
- Dixon JL, Ginsberg HN: Regulation of hepatic secretion of apolipoprotein-B containing lipoprotein. Information obtained from cultured liver cells. *J Lipid Res* 34: 167-79, 1993.
- Hahn SE, Goldberg DM: Factors affecting the regulation of apo B secretion by liver cells. *J Clin Lab Anal* 9: 431-49, 1995.
- Sparks JD, Sparks CE: Insulin regulation of triacylglycerol rich lipoprotein synthesis and secretion. *Biochem Biophys Acta* 1215: 9-32, 1994.
- Vance JE, Vance DE: Lipoprotein assembly and secretion by hepatocytes. *Annu Rev Nutr* 10: 337-56, 1990.
- Yao Z, McLeod RS: Synthesis and secretion of hepatic apolipoprotein B-containing lipoproteins. *Biochem Biophys Acta* 1212: 152-66, 1994.
- Brindle NPJ, Ontko JA: Suppression of triglyceride secretion by epinephrine in isolated rat hepatocytes. *Biochem Biophys Res Comm* 141(1): 191-7, 1986.
- Brindle NPJ, Ontko JA:  $\alpha_1$ -Adrenergic suppression of VLDL triacylglycerol secretion by isolated rat hepatocytes. *Biochem J* 250: 363-368, 1988.
- Rasouli M, Zahraei M:  $\alpha_1$ -Adrenergic suppression of VLDL-triacylglycerol and phospholipid secretion from isolated rat hepatocytes. Fourth Biochem Congress, Babol Univ., I.R. Iran, 75(Abs.), 1997.
- Bjornsson OG, Sparks JD, Sparks CE, Gibbons GF: Prostaglandins suppress VLDL secretion in primary rat hepatocyte cultures: relationships to hepatic calcium metabolism. *J Lipid Res* 33: 1017-27, 1992.
- Nossen Jø, Rustan AC, Drevon CA: Calcium-antagonists inhibit secretion of VLDL from cultured rat hepatocytes. *Biochem*
- Kwong TC, Sparks JD, Pryce DJ, Cianci JF, Sparks CE: Inhibition of apolipoprotein-B net synthesis and secretion from cultured rat hepatocytes by the calcium-channel blocker diltiazem. *Biochem J* 263: 411-5, 1989.
- Bjornsson OG, Duerden JM, Bartlet SM, Sparks JD, Sparks CE, Gibbons GF: The role of pancreatic hormones in the regulation of lipid storage, oxidation and secretion in primary cultures of rat hepatocytes. *Biochem J* 281: 381-6, 1992.
- Pullinger CR, Gibbons GF: Effects of hormones and pyruvate on the rates of secretion of VLDL triacylglycerol and cholesterol by rat hepatocytes. *Biochem Biophys Acta* 833: 44-51, 1985.
- Bjornsson OG, Sparks JD, Sparks CE, Gibbons GF: Regulation of VLDL secretion in primary culture of rat hepatocytes: involvement of cAMP and cAMP-dependent protein kinases. *Eur J Clin Invest* 24: 137-48, 1994.
- Klausner H, Soler-Argilaga C, Heimborg M: Effects of dibutyryl-cAMP on hepatic metabolism of free fatty acids. *Metab* 27(1): 13-25, 1978.
- Edwards PA, Lemongello D, Fogelman AM: The effect of glucagon, norepinephrine and dibutyryl cyclic-AMP on cholesterol efflux and on the activity of HMG-CoA reductase in rat hepatocytes. *J Lipid Res* 20: 2-7, 1979.
- Tsujimoto A, Tsujimoto G, Azhar S, Hoffman BB: Altered responsiveness to alpha- and beta-adrenoceptor stimulation in hepatocytes cultured in defined medium. *Biochem Pharm* 35(8): 1400-4, 1986.
- Sanghera JS, Vance DE: Stimulation of CTP: phosphocholine cytidyltransferase and phosphatidylcholine synthesis by calcium in rat hepatocytes. *Biochem Biophys Acta* 1003: 284-92, 1989.
- Pollard AD, Brindley DN: Effects of vasopressin and corticosterone on fatty acid metabolism and on the activities of glycerolphosphate acyltransferase and PAP in rat hepatocytes. *Biochem J* 217: 461-9, 1984.
- Roitelman J, Bar-Nun S, Inove S, Simoni RD: Involvement of calcium in the mevalonate-accelerated degradation of HMG-CoA reductase. *J Biol Chem* 266(24): 16085-91, 1991.
- Kosugi K, Harano Y, Nakano T, Suzuki M, Kashiwagi A, Shigeta Y: Mechanism of adrenergic stimulation of hepatic ketogenesis. *Metab* 32(1): 1081-7, 1983.
- Lodish HF, Kong N: Perturbation of cellular calcium blocks exit of secretory proteins from the rough endoplasmic reticulum. *J Biol Chem* 265(19): 10893-9, 1990.
- Stein O, Stein Y: Colchicine-induced inhibition of VLDL release by rat liver *in vivo*. *Biochem Biophys Acta* 306: 142-7, 1973.
- Rasouli M, Haghighi B, Suzangar M: Diurnal variation in hepatic and plasma lipids and in the activities of hepatic PAP and heart LPL. *Iran J Med Sci* 16(1): 46-53, 1991.
- Rasouli M, Zahraei M: Epinephrine suppresses VLDL-triacylglycerol secretion and increases triacylglycerol and phospholipid contents in isolated rat hepatocytes. *Med J Islam Rep Iran* (In Press).
- Folch J, Lees M, Stanley GHS: A simple method for the isolation and purification of lipids from animal tissues. *J Biol Chem* 226: 497-509, 1957.
- Mangiapan EH, Brindley DN: Effects of dexamethasone

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- and insulin on the synthesis of triacylglycerol and phosphatidylcholine in cultures of rat hepatocytes. *Biochem J* 233: 151-60, 1986.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin-phenol reagent. *J Biol Chem* 193: 265-75, 1951.
  30. Schatzman RC, Wise BC, Kuo JF: Phospholipid-sensitive calcium-dependent protein kinase: inhibition by anti-psychotic drugs. *Biochem Res Comm* 98(3): 669-76, 1981.
  31. Cohen P: The calmodulin-dependent multiprotein kinases. In: Cohen P, Klee CR. (eds.), *Calmodulin, Molecular Aspects of Cellular Regulation*. Netherlands: Elsevier Science, Vol. 5, pp. 145-187, 1988.
  32. Charest R, Blackmore PF, Berthon B, Exton JH: Changes in free cytosolic  $Ca^{2+}$  in hepatocytes following  $\alpha_1$ -adrenergic stimulation. *J Biol Chem* 258(14): 8769-73, 1983.
  33. Bode HP, Netter KJ: Agonist-releasable intracellular calcium stores and the phenomenon of store-dependent calcium entry. *Biochem Pharm* 51: 993-1001, 1996.
  34. Haghighi B, Rasouli M, Suzangar M: Inhibitory effect of epinephrine on PAP activity in isolated rat hepatocytes. *Rev Roum Endocrinol* 28: 149-54, 1990.
  35. Bygrave FL, Roberts HR: Regulation of cellular calcium through signaling cross-talk involves an intricate interplay between the actions of receptors, G-proteins, and second messengers. *FASEB J* 9: 1297-303, 1995.
  36. Wakelam MJO, Murphy GJ, Hruby VJ, Houslay MD: Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature* 323: 68-72, 1986.
  37. Combettes L, Berthon B, Binet A, Claret M: Glucagon and vasopressin interactions on  $Ca^{2+}$  movements in isolated hepatocytes. *Biochem J* 237: 675-83, 1986.