Type of dietary fat alters rat liver cell responsiveness to vasopressin

William L. Blake and Steven D. Clarke

The objective of this work was to determine if rat liver cell responsiveness to the phosphoinositide cascade agonist vasopressin was influenced by the source of dietary fat. Male Sprague-Dawley rats (185–200 g) were maintained for 21 days on a diet containing 20% metabolizable energy as safflower oil, tripalmitin, or menhaden fish oil. Freshly isolated rat hepatocytes were maintained for 20 minutes in a suspension media that contained 0, 0.1, 0.25, 0.5, 1.0, or 10 nmol/L arginine-vasopressin. Hepatocyte responsiveness to vasopressin was assessed by the activation of glycogen phosphorylase a *activity and glucose output. Dietary fat significantly* ($P < 0.05$) *altered hepatocyte vasopressin responsiveness. Relative to hepatocytes isolated from rats adapted to the safflower oil diet, hepatocytes from rats fed the menhaden oil or tripalmitin diets displayed a significant* ($P < 0.05$) *reduction in vasopressin-mediated glycogen phosphorylase activation and a reduction* ($P < 0.05$) *in hepatocyte glucose production. Stimulation of glycogen phosphorylase* a *activity and the glucose output by vasopressin did not differ between the tripalmitin and menhaden oil dietary groups. The level of glycogen phosphorylase* a *activity in non-vasopressin treated cells was unaffected by type of dietary fat. Our data indicate that depletion of* (n-6) *fatty acids from membrane phospholipids is associated with reduced hepatic responsiveness to vasopressin.*

Keywords: vasopressin; rats; hepatocytes; fish oil; glycogen phosphorylase

Introduction

Several metabolic processes are regulated by dietary fat, particularly fats rich in polyenic fatty acids.[1-4] For example, polyenic fatty acids inhibit expression of genes coding for lipogenic enzymes[1,2]; suppress triglyceride synthesis via inhibition of diacylglycerol acyltransferase[3,4]; and induce peroxisomal fatty acid oxidation.[5] These metabolic changes may be the direct consequence of feedback regulation by fatty acids, or the result of more indirect actions such as modifications of hormone sensitivity.[6-9] With respect to hormone action, dietary fats rich in long chain polyenic fatty acids have been reported to enhance epinephrine binding to membranes,[8] increase glucagon-stimulated adenylate cyclase activity, and improve peripheral tissue insulin sensitivity.[9] These three hormones are important regulators of hepatic fatty acid metabolism, and may function interactively with dietary polyenic fatty acids to regulate fatty acid partitioning between oxidation and triglyceride synthesis. Recent evidence indicates that vasopression enhances the catalytic efficiency of phosphatidic acid phosphohydrolase,[10] and in this way plays an important role as a regulator of hepatic triglyceride synthesis and triglyceride output.[10]

Since the cellular transducing events for the vasopressin signal involve several membrane dependent functions such as phosphoinositide metabolism, phospholipase C, protein kinase-C activation, and Ca-release, it seemed reasonable to hypothesize that enrichment of membrane phospholipid with 20- and 22-carbon (*n*-3) fatty acids brought about by ingestion of dietary fish oil would reduce the hepatocyte responsiveness to vasopressin, and thereby decrease the hepatic triglyceride synthetic rate and triglyceride output. This hypothesis was examined by quantifying vasopressin responsiveness in hepatocytes isolated from rats fed diets containing lipids rich in (*n*-3), (*n*-6), or saturated fatty acids.[4]

Materials and methods

Materials. Sprague-Dawley rats were obtained from the Upjohn colony. The fat-free basal diet (catalog # 15750) and safflower oil were purchased from US Biochemical (Cleveland, OH). Menhaden fish oil was obtained from Zapata Haynie Corporation (Reedsville, VA). Tripalmitin, arginine-vasopressin, and glucose-1-phosphate were purchased from Sigma Chemical (St. Louis, MO). U-^{14}-C-glucose-1-phosphate was pur-

Address reprint requests to S.D. Clarke at The Upjohn Company, Unit of Reproduction and Growth Physiology, Bldg. 25 Lab. 418, Kalamazoo, MI 49001, USA.
This work was partially supported by NIH grant R01 DK 39302.
Received April 18, 1990; accepted August 8, 1990.

© 1991 Butterworth–Heinemann

chased from New England Nuclear (Boston, MA). Collagenase (EC 3.4.24.3) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN).

General animal handling. Male Sprague-Dawley rats (185–200 g) were trained to a meal-eating regimen (0830–1200 h). Prior to hepatocyte isolation, the rats were maintained for 21 days on a high carbohydrate diet (sucrose, 58.43%; vitamin-free casein, 21.1%; celufil, 16.45%; salt mix USP XVII, 4.00%; and vitamin mix #23431 0.02%) supplemented (w/w) with: (a) 20% tripalmitin, (b) 10% safflower oil, or (c) 10% fish oil. Tripalmitin was provided at twice the oil amounts because its digestibility is only 50% that of the oils.[11,12] As a precaution against lipid peroxidation, the fat-free basal diet was supplemented with 0.1% butylated hydroxytoluene and the fats were mixed with the diet daily. The effect of feeding these lipids on liver fatty acid composition has been extensively characterized.[4,11] Consequently, determining hepatic fatty acid composition within this study was not deemed necessary.

Hepatocyte isolation. Rat hepatocytes were isolated by a modification of Seglen's procedure[13] as described by Salati and Clarke.[14] Hepatocytes isolated by this procedure have been found to be responsive to physiological concentrations of insulin, glucagon, growth hormone, and vasopressin,[15] and have been determined to maintain physiological levels of ATP during the incubation period.[15] Rats were anesthetized with sodium-pentobarbital and the portal vein cannulated. The lever was immediately perfused (40 mL/min) with oxygenated calcium-free buffer consisting of 142 mmol/L sodium chloride, 6.7 mmol/L potassium chloride, 20 mmol/L Hepes (pH 7.4), 25 mmol/L glucose, and 1 mmol/L EGTA. After 9 minutes, the liver was perfused (30 mL/min) with buffer containing collagenase (67 mmol/L sodium chloride, 25 mmol/L glucose, 6.7 mmol/L potassium chloride, 100 mmol/L Hepes pH 7.4, 6.3 mmol/L calcium chloride, and 0.5 mg collagenase/mL. After 10–12 minutes of perfusion, the liver was removed from the body cavity to a petri dish where the cells were combed free from the capsule. Hepatocytes were pelleted at 50*g* for 3 minutes and washed 3 times with Krebs/Henseleit buffer. Liver cells were diluted to a final concentration of 25 million cells/mL. Cell viability was determined by trypan blue exclusion and cellular ATP concentrations.[16]

Hepatocyte responsiveness to vasopression. The objective was to determine if hepatocyte vasopression responsiveness varied with source of dietary fat. Isolated hepatocytes were preincubated for 10 minutes at 37°C to equilibrate cells with the suspension media prior to the addition of hormone and substrate. Following preincubation, hepatocytes were allocated to 6 suspension flasks, each flask containing Krebs/Henseleit buffer, 2 mmol/L lactate/pyruvate (9:1 molar ratio), and vasopression (0, 0.1, 0.25, 0.5, 1.0, and 10 nmol/L). Final cell density was 5 million cells/mL in a total volume of 15 mL. Hepatocyte suspensions were incubated with treatments for 20 minutes at 37°C. Cell aliquots (1 mL) were removed at 0 and 5 minutes of incubation with the respective treatments and immediately frozen in liquid nitrogen for later assay of glycogen phosphorylase *a* activity.[17] Cell samples for glucose analysis were removed from the treatment flasks at 0, 10, and 20 minutes of incubation. The cells were pelleted and the supernatants removed for determination of glucose.[16] The data were subjected to a repeated measures analysis of variance and the means were compared by LSD.[18]

Results

The level of safflower oil utilized in these studies was sufficient to fulfill the essential fatty acid needs of the growing rat.[19] Therefore, for the purpose of these studies the safflower oil group was considered the reference group. Exchanging the safflower oil component of the diet with menhaden fish oil or tripalmitin significantly decreased hepatocyte vasopressin responsiveness ($P < 0.05$) as determined by reduced glycogen phosphorylase activation and less glucose release (*Figures 1* and *2*). Estimates of an EC 50 dose for vasopressin based on the curves in *Figures 1* and *2* indicate that the effect of tripalmitin and fish oil was to decrease the maximal response to vasopressin. For example, 10 nmol/L vasopressin (an apparent saturating dose) resulted in a glucose output in the (*n*-3) en-

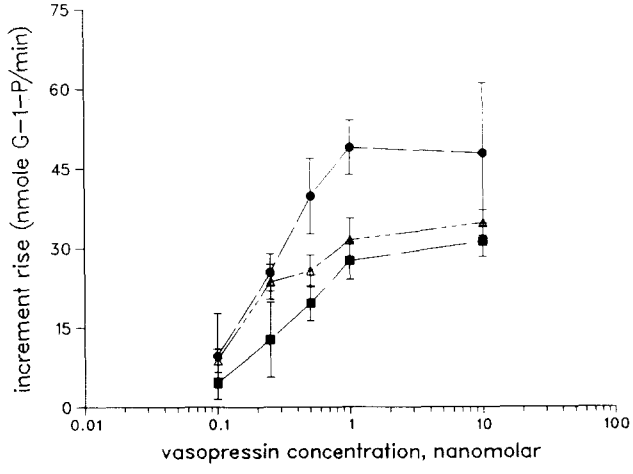

Figure 1 Vasopressin-mediated activation of glycogen phosphorylase *a* in hepatocyte suspensions isolated from rats fed safflower oil, fish oil, or tripalmitin. The graph depicts the increment rise in phosphorylase *a* activity induced by the cited concentrations of vasopressin. Hepatocytes ($n = 4$ preps/curve) were isolated from rats fed a diet containing safflower oil (solid circles), tripalmitin (open triangles), or menhaden oil (solid squares). Basal glycogen phosphorylase *a* activity in non-vasopressin treated hepatocytes was 32 ± 4, 35 ± 2, and 28 ± 5 nmol G-1-P incorporated into glycogen per min/10^6 cells for the safflower oil, tripalmitin, and menhaden oil groups, respectively. Data were subjected to repeated measures of analysis of variance and means were compared by least squares difference.[18] The effect of fat, vasopressin, and a fat × vasopressin interaction were significant ($P < 0.05$). The concomitant effect of vasopressin treatment on glucose production in these cell preparations is depicted in *Figure 2*.

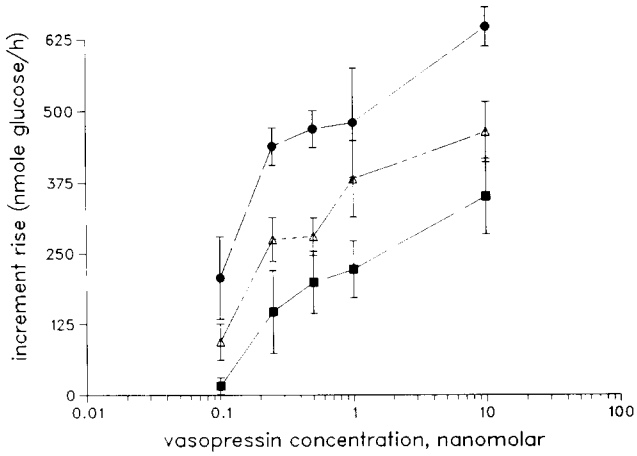

Figure 2. Vasopressin-mediated stimulation of glucose output by hepatocytes isolated from rats fed safflower oil, fish oil, or tripalmitin. The graph depicts the increment rise in glucose output induced by vasopressin. Hepatocytes ($n=4$ preps/curve) were isolated from rats fed a diet containing safflower oil (solid circles), tripalmitin (open triangles), or menhaden fish oil (solid squares). Values for the basal rate of glucose output in non-vasopressin treated hepatocytes were 252 ± 30, 373 ± 42, and 308 ± 72 nmol/h per 10^6 cells for the safflower oil, tripalmitin, and menhaden oil groups, respectively. Values are expressed as means ± SEM. Analysis of variance for repeated measures revealed a significant effect of fat and vasopressin ($P < 0.05$). Glycogen phosphorylase activation by vasopressin in these cell preps is depicted in *Figure 1*.

Table 1 Effect of dietary fat on basal activity of glycogen phosphorylase *a* and glucose production in rat hepatocytes

	Source of dietary fat		
	Safflower oil	Tripalmitin	Menhaden oil
Phosphorylase *a*,[1] (nmol G-1-P/min/10^6 cells)	32 ± 4	35 ± 2	28 ± 5
Glucose,[1] (nmol/h/10^6 cells)	252 ± 30	373 ± 42	308 ± 72

[1] Values are expressed as means ± SEM, n = 3–5. ANOVA revealed no significant effect ($P > 0.05$) of fat.

riched liver cells which was 300 nmol/h less than in the safflower oil group. Vasopressin-mediated activation of hepatocyte glycogen phosphorylase *a* and glucose output did not differ between the menhaden oil and tripalmitin dietary groups. In addition, basal glycogen phosphorylase *a* activity in isolated hepatocytes was not affected by the type of dietary fat ingested by the donor rat (*Table 1*).

Discussion

The fatty acid composition of membrane phospholipid in hepatocytes isolated from rats fed a comparable diet to that utilized in this study and containing safflower oil, menhaden fish oil, or saturated fat (hydrogenated coconut oil) has been exhaustively characterized.[4] When safflower oil was replaced with menhaden oil, hepatocyte membrane phospholipid 18:2 and 20:4 (*n*-6) were displaced 50–85% and 30–65% (dependent on phospholipid class), respectively, by the insertion of 20:5 and 22:5 (*n*-3) fatty acids.[4] Similarly, replacing safflower oil with a saturated fat led to a 2- and 6-fold enrichment in 18:1 and 20:3 (*n*-9) content of membrane phospholipid. This enrichment was accompanied by a 65% and 30% depletion of 18:2 and 20:4 (*n*-6), respectively.[4] We have hypothesized that these changes in membrane phospholipid fatty acid composition lead to altered hormonal responses within the hepatocyte. The hormone model chosen for study was vasopressin because it utilizes several lipid mediated events in the phosphoinositide cascade mechanism for hormonal signal transduction.[20-23]

Our data clearly indicate that activation of hepatocyte glycogen phosphorylase by vasopressin was dependent upon the type of dietary fat ingested. Specifically depleting rat liver cell membrane phospholipid of (*n*-6) fatty acids by feeding a diet low in (*n*-6) fatty acids or rich in (*n*-3) fatty acids was associated with a reduction in vasopressin-mediated activation of glycogen phosphorylase. The mechanism by which dietary fats modulate glycogen metabolism has several potential loci including vasopressin receptor binding, hormonal signal transduction,[6,24] and endoplasmic reticulum calcium release.[25] Regardless of the mechanism, it is important to emphasize that the reduced hepatocyte vasopressin sensitivity associated with dietary fish oil was not a unique feature of the high intake of (*n*-3) fatty acids, but rather was an outcome of the depletion of (*n*-6) fatty acids from the membrane phospholipid. Thus, certain metabolic events attributed to the intake of menhaden fish oil may reflect the displacement of long chain (*n*-6) fatty acids from the cell rather than a specific event mediated by (*n*-3) fatty acids. Finally, our data support the concept that hormones which utilize the phosphoinositide cascade as a signal transducing mechanism may be regulated by the type of dietary fat consumed. An extension of this conclusion is that vasopressin sensitivity of hepatocytes does not play a role in the antilipogenic mechanism of dietary polyunsaturated fats. This speculation is based upon the fact that tripalmitin, which reduced vasopressin sensitivity, has no ability to suppress fatty acid or triglyceride synthesis.[4,12]

Acknowledgments

The authors wish to thank K.A. Ash and L.F. Krabill for consultation on the statistical analysis of the data, and W.L. Buckner for preparation of the manuscript.

References

1 Clarke, S.D., Armstrong, M.K., Jump, D.B. (1990). Dietary polyunsaturated fats uniquely suppress rat liver fatty acid synthase and S14 mRNA content. *J. Nutr.* **120**, 225–231

2 Tomlinson, J.E., Nakayama, R., Holten, D. (1988). Repression of pentose phosphate pathway dehydrogenase synthesis and mRNA by dietary fat in rats. *J. Nutr.* **118**, 408–415

3 Rustan, A.C., Nossen, J.O., Christiansen, E.N., Drevon, C.A. (1988). Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of

acyl-coenzyme A; 1,2-diacylglycerol acyltransferase. *J. Lipid Res.* **29,** 1417–1426
4 Strum-Odin, R., Adkins-Finke, B., Blake, W.L., Phinney, S.D., Clarke, S.D. (1987). Modification of fatty acid composition of membrane phospholipid in hepatocyte monolayer with *n*-3, *n*-6, and *n*-9 fatty acids and its relationship of triacylglycerol production. *Biochim. Biophys. Acta* **921,** 378–391
5 Yamazaki, R.K., Shen, T., Schade, G.B. (1987). A diet rich in (*n*-3) fatty acids increase peroxisomal B-oxidation activity and lowers plasma triacylglycerols without inhibiting glutathione-dependent detoxication activities in the rat liver. *Biochim. Biophys. Acta* **920,** 62–67
6 Lee, C., Hamm, M.W. (1989). Effect of dietary fat and cholesterol supplements on glucagon receptor binding and adenylate cyclase activity of rat liver plasma membrane. *J. Nutr.* **119,** 539–546
7 Salati, L.M., Adkins-Finke, B., Clarke, S.D. (1988). Free fatty acid inhibition of the insulin induction of glucose-6-phosphate dehydrogenase in rat hepatocyte monolayers. *Lipids* **23,** 36–41
8 Ham, M.W., Shei, G. (1988). Dietary lipid, adenylate cyclase activity, and B-adrenergic binding in rat hearts. *FASEB J.* **2,** A639 (abstract)
9 Storlien, L.H., Kraegen, E.W., Chisolm, D.J., Ford, G.L., Bruce, D.G., Pascoe, W.S. (1987). Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* **237,** 885–888
10 Pollard, A.D., Brindley, D.N. (1984). Effects of vasopressin and corticosterone on fatty acid metabolism and on the activities of glycerol phosphate and acyltransferase and phosphatidate phosphohydrolase in rat hepatocytes. *Biochem. J.* **217,** 461–469
11 Clarke, S.D., Wilson, M.D., Ibnoughazala, T. (1984). Resistance of lung fatty acid synthesis to inhibition by dietary fat in the meal-fed rat. *J. Nutr.* **114,** 598–605
12 Clarke, S.D., Romsos, D.R., Leveille, G.A. (1977). Differential effects of dietary methylesters of long-chain saturated and polyunsaturated fatty acids on rat liver and adipose tissue lipogenesis. *J. Nutr.* **107,** 1170–1181
13 Seglen, P.O. (1972). Preparation of rat liver cells. *Exp. Cell Res.* **74,** 450–454
14 Salati, L.M., Clarke, S.D. (1986). Fatty acid inhibition of hormonal induction of acetyl-coenzyme A carboxylase in hepatocyte monolayers. *Arch. Biochem. Biophys.* **246,** 82–89
15 Blake, W.L., Clarke, S.D. (1989). Somatotropin acutely increases glucose output by hepatocytes isolated from hypophysectomized rats. *J. Endocrinology* **122,** 457–464
16 Lowry, O.H., Passoneau, J.V. (1972). A collection of metabolic assays. In: *A Flexible System of Enzymatic Analysis*. P. 174. Academic Press, New York
17 Blackmore, P.F., Exton, J.H. (1985). Assessment of effects of vasopressin, angiotensin II and glucagon on Ca fluxes and phosphorylase activity in liver. *Meth. Enzymol.* **109,** 550–558
18 SAS Institute. (1985). *SAS User's Guide*. SAS Institute, Inc., Cary, NC
19 Mohrhauer, H., Holman, T.R. (1963). The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *J. Lipid Res.* **4,** 151–159
20 Berridge, M.J. (1987). Inositol trisphosphate and diacylglycerol: Two interacting second messengers. *Ann. Rev. Biochem.* **56,** 159–193
21 Williamson, J.R., Cooper, R.H., Joseph, S.K., Thomas, A.P. (1985). Inositol trisphosphate and diacylglycerol as intracellular second messengers in liver. *Amer. J. Physiol.* **248,** C203–216
22 Chrisman, T.D., Jordan, J.E., Exton, J.H . (1982). Purification of rat liver phosphorylase kinase. *J. Biol. Chem.* **257,** 10798–10804
23 Hems, D.A., Rodrigues, L.M., Whitton, P.D. (1976). Glycogen phosphorylase, glucose output and vasoconstriction in the perfused rat liver. *Biochem. J.* **160,** 367–374
24 Tsai, M., Yu, C., Wei, F., Stacey, D.W. (1989). The effect of GTPase activating protein upon Ras is inhibited by mitogenically responsive lipids. *Science* **243,** 522–525
25 Swason, J.E., Lokesh, B.R., Kinsella, J.E. (1989). Ca-Mg ATPase of mouse cardiac sarcoplasmic reticulum is affected by membrane *n*-6 and *n*-3 polyunsaturated fatty acid content. *J. Nutr.* **119,** 364–372