

OPEN ACCESS

Full open access to this and thousands of other papers at <http://www.la-press.com>.

Prolonged Treatment with Free Fatty Acids has Post Receptor Effect in Hepatic Insulin Resistance: Evidence that Fatty Acids, Oleate and Palmitate have Insignificant Effect on the Insulin Receptor Beta *In Vivo* and *Ex Vivo* Primary Hepatocytes

Rafik Ragheb^{1,2,3,4,5}, Amina M. Medhat¹, Gamila M.L. Shanab¹, Dina M. Seoudi¹ and Fantus I.G.^{2,3,5}

¹University of Ain Shams, Department of Biochemistry, Faculty of Science, Cairo, Egypt. ²University Health Network,

³Mount Sinai Hospital, ⁴Hospital for Sick Children, ⁵University of Toronto, Toronto, Canada.

Email: rafik.ragheb@utoronto.ca

Abstract: In the current study, we used immunoprecipitation and immunoblotting to examine the levels and phosphorylation status of the insulin receptor-beta subunit (IR- β), as well as the down stream target in PI3K pathway, total PKB/Akt as well as their phosphorylated forms. The assessment of FFAs treatment showed no direct and significant effect on the PI3K stimulation, specifically the IR- β in primary hepatic control cells treated with insulin. Cells treated with either oleate or palmitate (360 μ M) showed no statistically significant values following insulin stimulation ($P > 0.05$). To further investigate the effect of both FFAs and high insulin (1 μ g), we examined the effects of oleate and palmitate at 360 μ M concentration on IR- β as well as PKB. There was no significant difference in the total protein levels and their phosphorylated forms in cells treated with or without oleate or plamitate. Interestingly, IR- β tyrosine phosphorylation showed a similar insignificant effect *in vivo* and *ex vivo* hepatic cells treated with oleate or palmitate in comparison to their controls in the fructose fed hamsters.

Keywords: obesity, free fatty acids, hepatic insulin resistance, insulin signaling

Biochemistry Insights 2009:2 29–36.

This article is available from <http://www.la-press.com>.

© the authors, licensee Libertas Academica Ltd.

This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://www.creativecommons.org/licenses/by/2.0>) which permits unrestricted use, distribution and reproduction provided the original work is properly cited.



Introduction

The close association between the ongoing worldwide epidemic of obesity, insulin resistance and type 2 diabetes has been well documented.^{1–5} Free fatty acids (FFA) have been implicated as an important causative factor in the development of type 2 diabetes.⁶ The FFA-induced impairment of glucose metabolism in muscle is well documented,^{7–15} but little is known about the mechanisms of the FFA-induced impairment of glucose metabolism and the impairment of the insulin signaling cascade in the liver.^{12,16}

In the liver, FFA increase gluconeogenesis both *in vitro* and *in vivo*.^{1,16,17} A large number of studies have demonstrated that Intralipid with heparin increases endogenous glucose production during euglycemic clamps.^{7,18–22} Randle et al²³ have shown that FFA compete with glucose for substrate oxidation (the glucose-fatty acid cycle) demonstrating the mechanism of the FFA effect on hepatic insulin signaling. In addition, citrate-induced inhibition of phosphofructokinase-1, was observed in the perfused rat liver in isolated hepatocytes exposed to FFA.^{24–26} The hepatic insulin resistance in high-fat-fed rats was not ameliorated by etomoxir,¹² an inhibitor of fatty acid oxidation, suggesting that other mechanisms are likely involved in FFA-induced hepatic insulin resistance.

The present study was performed 1) to investigate, whether chronic treatment of hamster hepatocytes with FFA induces changes in insulin signaling and development of insulin resistance and 2) whether, chronic exposure of hamster hepatocytes with high dose of insulin and FFA leads to desensitization of the PI3K pathway.

Experimental Methods

Materials

Fetal bovine serum (certified grade), liver perfusion medium, hepatocyte wash medium, liver digest medium, and hepatocyte attachment medium were obtained from Life Technologies (Grand Island, NY). Fatty acid free bovine serum albumin, protein A-Sepharose, oleic acid and palmitate were from Sigma Chemical Co. (St. Louis, USA). IR- β antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). PKB total antibodies and phosphospecific phospho—Serine⁴⁷³ PKB and I κ B α were from Upstate Biotechnology (Lake Placid, NY).

Animal protocols

Male Syrian golden hamsters (*Mesocricetus auratus*) were obtained from Charles River Canada (Montreal, QC). All animals were placed on either the control diet (normal chow) or fructose-enriched diet (hamster diet containing 60% fructose, Dyets Inc., Bethlehem, PA). The diet was continued for 2 weeks and hamster weight was monitored every 2 days.²⁷

Liver perfusion and isolation of primary hamster hepatocytes

Male Syrian golden hamsters (*Mesocricetus auratus*) were purchased from Charles River (Montreal, QC). At the end of the 2-week feeding period, hamsters were fasted overnight and were anesthetized. Primary hepatocytes are digested by digest medium (Life Technologies). Liver cells were collected by gradient centrifugation (3 \times 5 min at 500 rpm). Hepatocytes were washed three times in hepatocyte wash medium and were resuspended in serum-free Hepatocyte Attachment Medium (Life Technologies, Inc.). After 4 h incubation at 37 °C, 5% CO₂, attached cells were used to carry out the experiments.²⁷

Lipid preincubation

Lipid containing media were prepared by conjugation of FFAs with BSA (4%), using a method modified from that described by Svedberg et al.²⁸ Briefly, FFAs were dissolved in ethanol and diluted 1:25 at 45 °C, each solution contained 20% (w/v) fatty acid-free bovine serum albumin. Solutions were filter-sterilized and diluted 1:4 with serum or serum free (SF-William's E) as appropriate. Control media were prepared similarly containing ethanol and BSA in the absence of lipid. Primary hepatocytes were incubated for 16 hrs in 10 mm dishes at 3 ml/well in the absence or presence of FFA.

Chemiluminescent immunoblot analysis

Treated cells were collected in lysing buffer (150 mM NaCl, 10 mM trishydroxymethyl amino ethane, pH = 7.4, 1 mM EDTA, 1 mM EGTA, 1% Triton \times 100, 1%NP-40, 2 mM PMSF, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin, 100 mM sodium flouride, 10 mM sodium pyrophosphate and 2 mM sodium orthovanadate). The total cell lysates were either used directly or after immunoprecipitation against a target protein,²⁹ were subjected to chemiluminescent



immunoblotting for IR- β (Santa Cruz), total PKB/Akt (Upstate) as well as their phosphorylated forms at serine 473. Following SDS-PAGE the proteins were transferred electrophoretically overnight at 4 °C onto nitrocellulose membranes or PVDF.³⁰ The immunoblots were probed with the indicated antiserum. Bands were visualized using an enhanced chemiluminescence detection reagent (Amersham Pharmacia Biotech), and exposed to Kodak Hyperfilm. Films were developed and quantitative analysis was performed using an Imaging Densitometer (FluorChem and Alpha Innotech Corporation).

Statistical methods

All results are expressed as means \pm SE, Statistical calculations were performed using Student's t test. A value is considered significant at $p < 0.05$.

Results

Effect of oleate or palmitate on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in control primary hamster hepatocytes

The monounsaturated FFA, oleate (18:1n9), and the saturated FFA, palmitate (16:0). Figure 1 shows the effect of oleate and palmitate on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in primary hepatocytes from chow-fed hamsters. We have examined the levels and phosphorylation status of the insulin receptor—beta subunit (IR- β), the down stream target in PI3K pathway, total PKB/Akt as well as their phosphorylated forms. Interestingly, oleate and palmitate treatment showed a negative effect on the PI3K stimulation in primary hepatic control cells treated with insulin. Cells treated with either oleate or palmitate showed no statistically significant values following insulin stimulation ($P > 0.05$).

Effect of oleate or palmitate (360 μ M) on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in control primary hamster hepatocytes in the presence of high insulin

To further investigate the effect of both FFA and high insulin levels (1 μ g/ml), we examined the effects of oleate and palmitate (360 μ M) on molecules of

the insulin-signaling pathway, IR- β as well as PKB. Figures 2A, B show the effect of FFAs on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in control primary hamster hepatocytes. There was no significant difference in the total protein levels in cells treated with or without oleate or palmitate. IR- β tyrosine phosphorylation showed a similar negative effect in the cells treated with oleate and palmitate in comparison with control cells stimulated with insulin.

Effect of oleate or palmitate on IR- β subunit tyrosine phosphorylation in fructose fed primary hamster hepatocytes

Figure 3 shows the effect of oleate or palmitate on the PI3-K pathway and insulin signaling cascade in primary hepatocytes from fructose fed hamster. Phosphorylation of the IR β subunit was assessed in cell lysates by first immunoprecipitating with the IR β subunit followed by blotting for tyrosine phosphorylation. There was no additional effect above basal insulin signaling/phosphorylation upon treatment with either oleate or palmitate (Fig. 3A). The mass of IR for the same experiment was also verified (Fig. 3B) and this showed no significant change in IR mass suggesting that the lack of effect of FFAs on the PI3K pathway and insulin signaling cascade was not due to changes in the IR mass. Each blot is a representation for one individual experiment.

Discussion

The majority of studies have focused on the acute effects of insulin, while the role of chronic hyperinsulinemia and free fatty acids in insulin resistance has been understudied. We employed a fructose-fed hamster as a model of insulin resistance to investigate the mechanisms involved in the state of insulin resistance. This model offers advantages over the more commonly used fructose-fed rat model, in that the metabolism of its apo-B-containing lipoproteins is more similar to that of humans. The rationale for the current study is testing the hypothesis that fructose-feeding results in FFA flux to the liver. Fatty acid esterification pathway rather than fatty oxidation could be implicated in hepatic insulin resistance. This will lead to accumulation of Diacylglycerol (DAG), Triacyl glycerol (TAG) and ceramides. As a result, FFA flux may desensitize

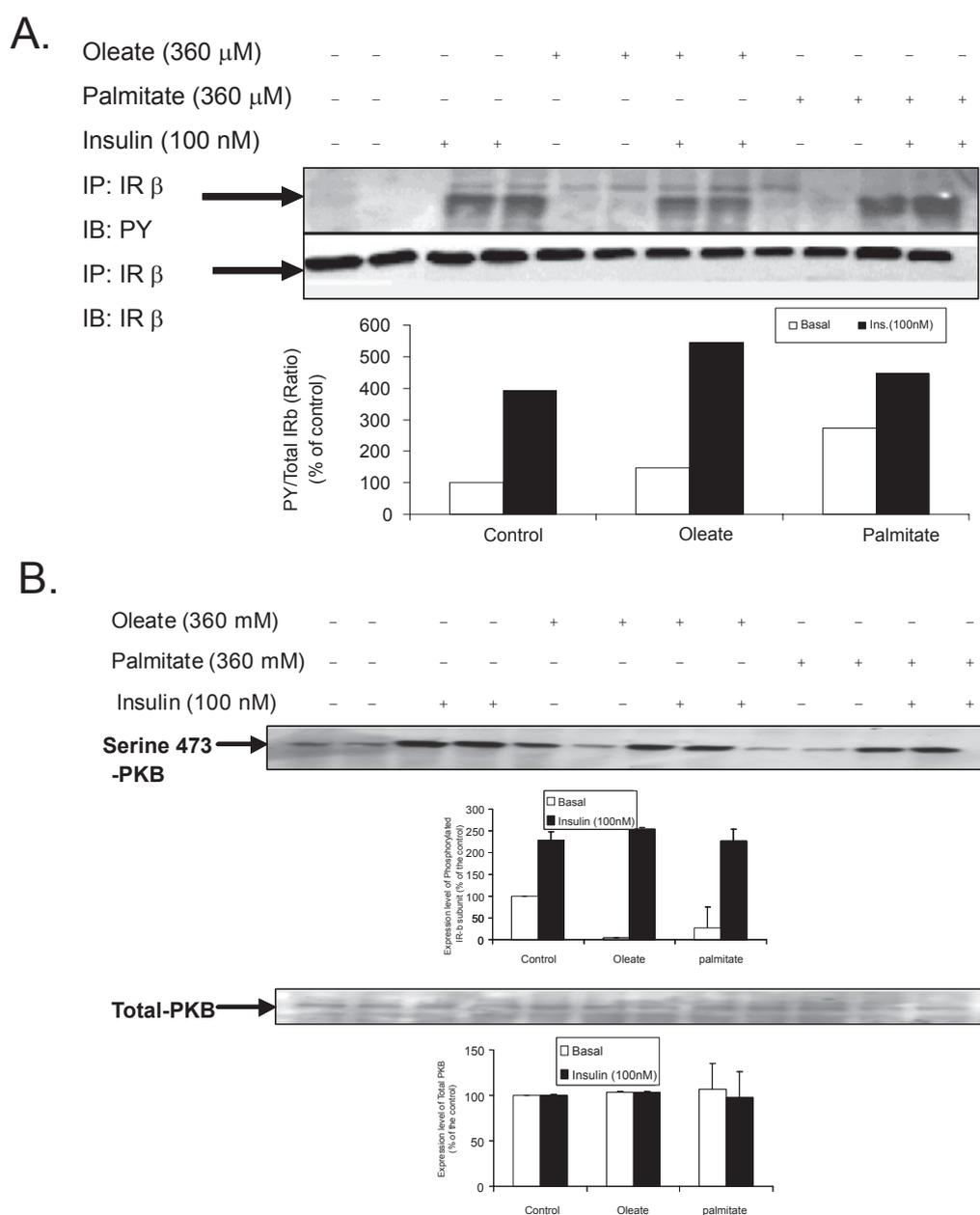


Figure 1. Effect of oleate or palmitate on IR- β subunit tyrosine phosphorylation phosphorylation in control primary hamster hepatocytes—Primary hamster hepatocytes from chow-fed hamsters were treated for 10 min with (100 nM) insulin and 360 μ M FFA for 16 hours. **(A)** Cells were lysed and immunoprecipitated using anti-IR β mouse monoclonal anti body IgG followed by immunoblot with antiphosphotyrosine PY (99) mouse monoclonal antibody. **(B)** Membranes were reprobed using anti IR- β to verify mass. Each blot is a representation for one individual experiment ($n = 3$ experiments/group). T-test was conducted to compare FFAs treated versus appropriate basal or insulin stimulated, significant difference from STD ($p < 0.05$).

hepatic cells to insulin action and reduce tyrosine phosphorylation of IR- β and other downstream signaling molecules involved in PI3K pathway.

In the present study, we intended to investigate, whether chronic treatment of hamster hepatocytes with FFA induces changes in insulin signaling and leads to the development of insulin resistance. It has been shown that liver is characterized as being one of the most insulin sensitive tissues in addition to muscle

and adipose tissues. FFA-induced impairment in insulin clearance has been demonstrated in previous *in vivo* studies.^{31–33}

Surprisingly, our data have shown insignificant effect seen for oleate and palmitate on total IR- β receptor mass as well as the IR- β tyrosine phosphorylation in comparison with controls upon stimulation with insulin. The results were confirmed by using a dose response (250 μ M–2000 μ M) that

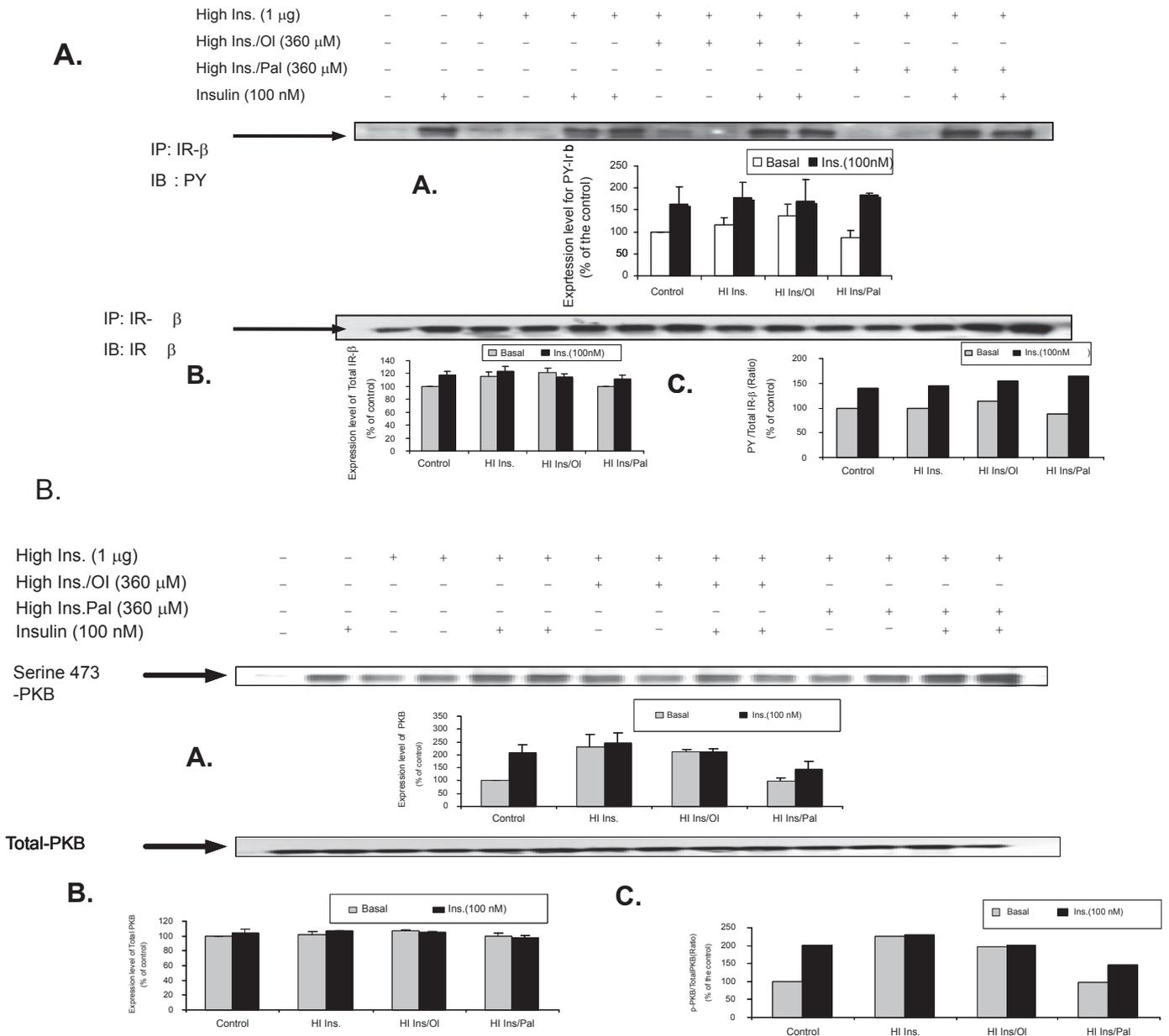


Figure 2. Effect of oleate or palmitate on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in control primary hamster hepatocytes in the presence of high insulin—Figure 2A, B show a representative immunoblot and graphical representation of the effect of FFA on IR- β subunit tyrosine phosphorylation and PKB phosphorylation in control primary hamster hepatocytes. Each blot is a representation for one individual experiment ($n = 3$ experiments/group). T-test was conducted to compare FFA. To further investigate the effect of both FFAs and high insulin (1 μ g/ml), we examined the effects of oleate and palmitate (360 μ M) on molecules of the insulin-signaling pathway, IR- β as well as PKB. There was no significant difference in the total protein levels in cells treated with or without oleate or palmitate. IR- β tyrosine phosphorylation showed a similar negative effect in the cells treated with oleate and palmitate in comparison with control cells stimulated with insulin. T-test was conducted to compare FFAs treated versus appropriate basal or insulin stimulated, significance difference from STD ($p < 0.05$).

revealed similar results using either oleate or palmitate (data not shown). Down stream of the IR- β , we also investigated the effect of both oleate and palmitate on the phosphorylation status of PKB. There was no significant effect seen for oleate and palmitate on total PKB mass as well as the serine 473 PKB phosphorylation status that showed a similar results

in comparison to their controls upon stimulation with insulin.

This led us to ask the second question: does chronic treatment of hamster hepatocytes with high insulin and FFA desensitize the PI3K pathway. Our data demonstrated that there was insignificant effect seen for oleate and palmitate on total IR- β receptor

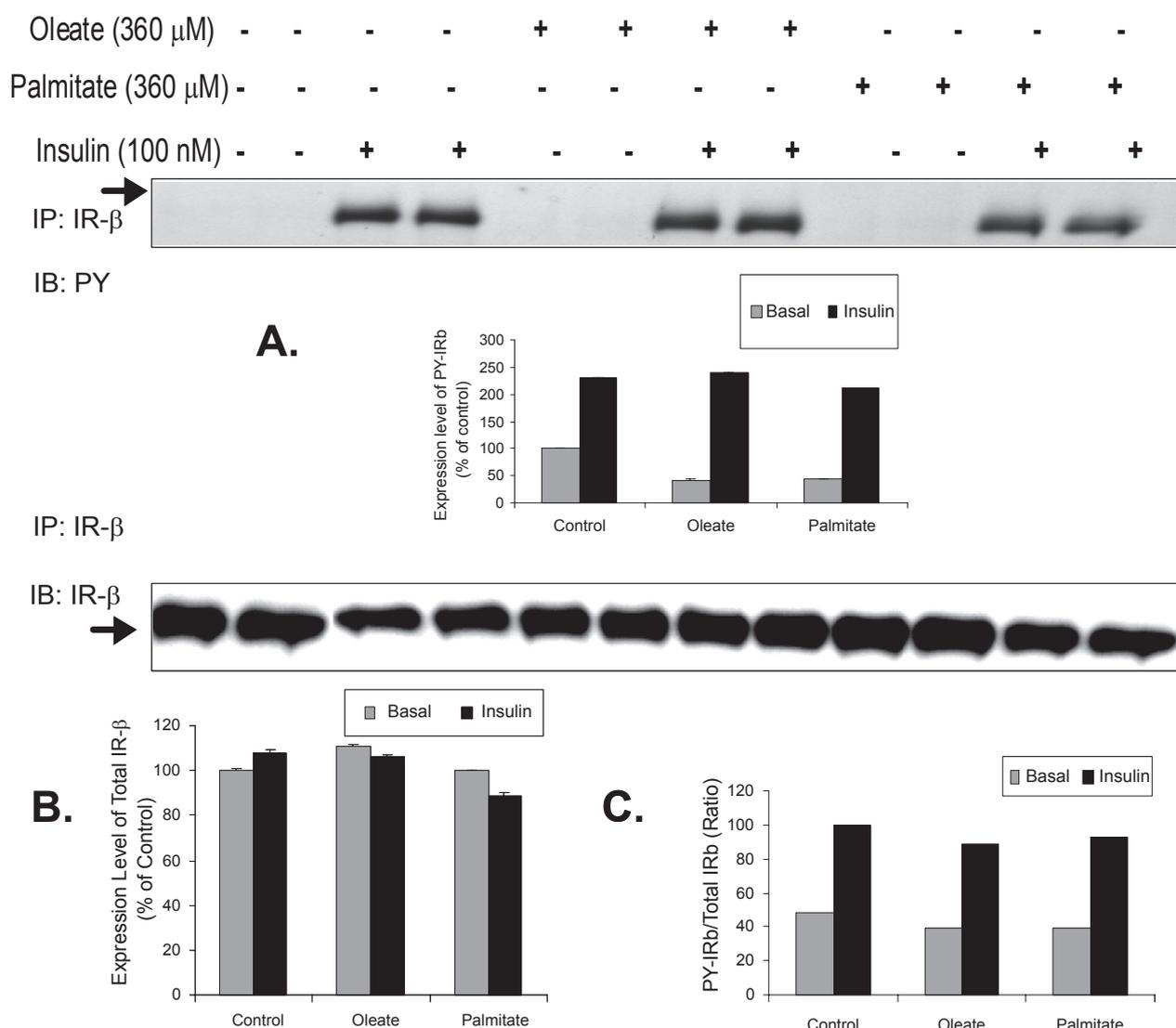


Figure 3. Effect of oleate or palmitate on IR- β subunit tyrosine phosphorylation in primary hamster hepatocytes from fructose-fed hamsters—Primary hamster hepatocytes from fructose-fed hamsters were treated for 10 min/hours with insulin and 360 μ M FFA for 16hours. (A) Cells were lysed and immunoprecipitated using anti-IR β mouse monoclonal antibody IgG followed by immunoblot with antiphosphotyrosine PY (99) anti mouse monoclonal antibody. (B) Membranes were reprobed using anti IR β to verify mass T-test was conducted to compare FFAs treated versus appropriate basal or insulin stimulated, significant difference from STD ($p < 0.05$), ($n = 3$ experiments/group).

mass as well as the IR- β tyrosine phosphorylation that showed a similar result in comparison with their controls upon stimulation with insulin. Down stream of the IR- β , we also investigated the effect of both oleate and palmitate on the phosphorylation status of PKB. There was no significant effect seen for oleate and palmitate at on total PKB mass as well as the serine 473 PKB phosphorylation status that showed similar results in comparison with controls observed upon stimulation with insulin. Interestingly, primary hepatocytes extracted from fructose fed hamster have demonstrated a similar effect to the FFA treatment

and showed insignificant effect response to the insulin stimulation.

Studies have demonstrated that the number of insulin receptors in liver cells is reduced in glucose-intolerant obese subjects.³⁵ High FFA levels in the portal blood are correlated with a decreased clearance of insulin in rat liver.³⁶ Previously, the effect of FFAs on insulin binding, degradation and action was examined in isolated rat hepatocytes. The effect of 0.4 mM oleic acid on insulin binding was reduced cell associated insulin binding by 45%. In agreement to our data, it was surprising to find, FFA does not affect the receptor



affinity (comparing cells exposed to oleic acid and their controls). Lonroth P et al demonstrated that partially purified insulin receptors from solubilized hepatocytes preincubated in the absence or presence of 0.2 mM oleic acid for 45 min with no difference in the binding.³⁷ It has been noted previously that the technique used for the hepatocytes preparation seems to be crucial for the kinetics of insulin binding and the insulin effect. It has also been demonstrated that the increase of oleic acid levels effectively reduced basal amino acid uptake and the maximal insulin effect, indicating the existence of post receptor perturbations.³⁸

Independent of the PI3K pathway, other mechanisms might be involved in the development of insulin resistance upon treatment with FFAs. Protein kinase C (PKC), a family of protein serine/threonine kinases has been implicated in FFA-induced insulin resistance.¹⁴ It was postulated that free fatty acids activate classical PKCs isozymes in pretreated muscle cells that lead to insulin resistance development.^{14,39} Similar *in vivo* studies conducted in the liver, have shown that FFA-induced impairment in insulin clearance.^{40,41} The mechanism for this impairment is likely a decrease in hepatocyte insulin binding caused in part by FFA-induced PKC activation.^{42,43} Other groups have demonstrated FFA-induced insulin resistance occur through different mechanisms that induce NF κ B activation.⁴⁴

The current findings substantiate our earlier published data which revealed that treatment of cells with FFAs—induced hepatic insulin resistance through the PKC-NF κ B activation pathway. Earlier we have reported that the phosphosite screen using the proteomics approach clearly showed a significant difference in the expression level of the key signaling components involved in the pathway. It manifested a significant increase in the phosphorylation of AMP-activated protein kinase alpha (α), I-kappa-B kinase beta, MKK6, PKC related kinases1 and 2, Raf as well as the mammalian target of rapamycin (mTOR). Consistent with these findings, and in support of our previous published data, a down regulation in the expression level of glycogen synthase kinase-3 alpha/beta (α/β), protein kinase B (PKB) and PKC zeta and lambda (ζ/λ) was observed. The data suggest that fructose feeding develops hepatic insulin resistance through a combination of mechanisms involving the

attenuation of the PI3K pathway through FFA effect that occurs down stream of IR- β .⁴⁵

Acknowledgements

Authors are grateful to Professor Dr. K. Adeli for his great efforts as a consultant expert in the field of insulin resistance and metabolic dyslipidemia for guiding and revising the manuscript. We also wanted to thank Dr. R.K. Avramoglu, for her sincere feedback and her technical advices during the conductance of the experimental work (The Hospital for Sick Children, Toronto, Canada).

Conflict of Interest

Rafik Ragheb is a recipient of the Hospital for Sick Children Research Training Competition Scholarship. (Restra-comp)—The University of Toronto, Canada.

Abbreviations

FFA, free fatty acid; Insulin Receptor-beta subunit (IR- β); PKB, protein kinase B.

References

1. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes*. 1997;46:3–10.
2. Henry RR. Impaired muscle fat metabolism: a cause or effect of visceral obesity? *J Clin Invest*. 1995;95:1427–8.
3. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes*. 2000;49:677–83.
4. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev*. 2002;23:201–29.
5. McGarry JD. Glucose-fatty acid interactions in health and disease. *Am J Clin Nutr*. 1998;67:500S–4.
6. Paolisso G, Tataranni PA, Foley JE, Bogardus C, Howard BV, Ravussin E. A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. *Diabetologia*. 1995;38:1213–7.
7. Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest*. 1994;93:2438–46.
8. Dresner A, Laurent D, Marcucci M, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest*. 1999;103:253–9.
9. Griffin ME, Marcucci MJ, Cline GW, et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C-theta and alterations in the insulin signaling cascade. *Diabetes*. 1999;48:1270–4.
10. Hawkins M, Barzilai N, Liu R, Hu M, Chen W, Rossetti L. Role of the glucosamine pathway in fat-induced insulin resistance. *J Clin Invest*. 1997;99:2173–82.
11. Jucker BM, Rennings AJ, Cline GW, Shulman GI. ¹³C and ³¹P NMR studies on the effects of increased plasma free fatty acids on intramuscular glucose metabolism in the awake rat. *J Biol Chem*. 1997;272:10464–73.
12. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. *Diabetes*. 1997;46:1768–74.
13. Park JY, Kim CH, Hong SK, Suh KI, Lee KU. Effects of FFA on insulin-stimulated glucose fluxes and muscle glycogen synthase activity in rats. *Am J Physiol Endocrinol Metab*. 1998;275:E338–44.



14. Roden M, Price TB, Perseghin G, Petersen KF, et al. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest.* 1996;97:2859–65.
15. Schmitz-Peiffer C, Browne CL, Oakes ND, et al. Alterations in the expression and cellular localization of protein kinase C isozymes epsilon and theta are associated with insulin resistance in skeletal muscle of the high-fat-fed rat. *Diabetes.* 1997;46:169–78.
16. Massillon D, Barzilai N, Hawkins M, Prus-Wertheimer D, Rossetti L. Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. *Diabetes.* 1997;46:153–7.
17. Williamson JR, Kreisberg RA, Felts PW. Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc Natl Acad Sci U S A.* 1966;56:247–54.
18. Bevilacqua S, Bonadonna R, Buzzigoli G, et al. Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. *Metabolism.* 1987;36:502–6.
19. Rebrin K, Steil GM, Mittelman SD, Bergman RN. Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J Clin Invest.* 1996;98:741–9.
20. Saloranta C, Koivisto V, Widen E, et al. Contribution of muscle and liver to glucose-fatty acid cycle in humans. *Am J Physiol Endocrinol Metab.* 1993;264:E599–605.
21. Sindelar DK, Chu CA, Rohlie M, Neal DW, Swift LL, Cherrington AD. The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes.* 1997;46:187–96.
22. Wiesenthal SR, Sandhu H, McCall RH, et al. Free fatty acids impair hepatic insulin extraction *in vivo*. *Diabetes.* 1999;48:766–74.
23. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* 1963;1:785–9.
24. McCune SA, Durant PJ, Jenkins PA, Harris RA. Comparative studies on fatty acid synthesis, glycogen metabolism, and gluconeogenesis by hepatocytes isolated from lean and obese Zucker rats. *Metabolism.* 1981;30:1170–8.
25. Morand C, Besson C, Demigne C, Remesy C. Importance of the modulation of glycolysis in the control of lactate metabolism by fatty acids in isolated hepatocytes from fed rats. *Arch Biochem Biophys.* 1994;309:254–60.
26. Williamson JR, Browning ET, Scholz R. Control mechanisms of gluconeogenesis and ketogenesis. I. Effects of oleate on gluconeogenesis in perfused rat liver. *J Biol Chem.* 1969;244:4607–16.
27. Taghibiglou C, Rashid-Kolvear F, Van Iderstine SC, et al. Hepatic Very Low Density Lipoprotein-ApoB Overproduction Is Associated with Attenuated Hepatic Insulin Signaling and Overexpression of Protein-tyrosine Phosphatase 1B in a Fructose-fed Hamster Model of Insulin Resistance. *J Biol Chem.* 2002;277:793–803.
28. Schmitz-Peiffer C, Craig DL, Biden TJ. Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *J Biol Chem.* 1999;274:34:24202–10.
29. Taghibiglou C, Rudy D, Van Iderstine SC, et al. *J Lipid Res.* 2000;41:499–513.
30. Laemmli UK. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature.* 1970;227:680–5.
31. Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol Endocrinol Metab.* 1999;276:E1055–66.
32. Hennes MM, Dua A, Kissebah AH. Effects of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes.* 1997;46:57–62.
33. Mittelman SD, Van Citters GW, Kim SP, et al. Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. *Diabetes.* 2000;49:2116–25.
34. Duckworth WC. Insulin degradation: mechanisms, products, and significance. *Endocr Rev.* 1988;9(3):319–45.
35. Arner P, Einarsson K, Ewerth S, Livingston J. Studies of the human liver insulin receptor in noninsulin-dependent diabetes mellitus. *J Clin Invest.* 1986;77(5):1716–8.
36. Stromblad G, Bjorntorp P. Reduced hepatic insulin clearance in rats with dietary-induced obesity. *Metabolism.* 1986;35(4):323–7.
37. Svedberg J, Bjorntorp P, Smith U, Lonnroth P. Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes.* 1990;39(5):570–4.
38. Ammeltoft S, Kristensen LO, Sestoft L. Insulin receptors in isolated rat hepatocytes. Reassessment of binding properties and observations of the inactivation of insulin at 37 degrees C. *J Biol Chem.* 1978;253(23):8406–13.
39. Kuranami M, Powell CT, Hug H, Zeng Z, Cohen AM, Guillem JG. Differential expression of protein kinase C isoforms in human colorectal cancers. *J Surg Res.* 1995;58:233–9.
40. Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol Endocrinol Metab.* 1999;276:E1055–66.
41. Hennes MM, Dua A, Kissebah AH. Effects of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes.* 1997;46:57–62.
42. Chen S, Wiesenthal SR, Lam L, et al. Giacca Oleate-induced decrease in hepatocyte insulin binding is mediated by protein kinase C activation. *Diabetes.* 1999;48 Suppl 1:A225.
43. Damiano P, Cavallero S, Mayer M, et al. Impaired response to insulin associated with protein kinase C in chronic fructose-induced hypertension. *Blood.* 2002;11(6):345–51.
44. Boden G, She P, Mozzoli M, et al. Free Fatty Acids Produce Insulin Resistance and Activate the Proinflammatory Nuclear Factor- κ B Pathway in Rat Liver. *Diabetes.* 2005;54(12):3458–65.
45. Ragheb R, Medhat AM, Shanab GML, Seoudi DM, Fantus IG. Links between enhanced Fatty Acid Flux, Protein Kinase C and NF κ B Activation, and ApoB-Lipoprotein Production in the Fructose Fed Hamster Model of Insulin Resistance. *Biochem Biophys Res Commun.* 2008;370(1):134–9.

Publish with Libertas Academica and every scientist working in your field can read your article

“I would like to say that this is the most author-friendly editing process I have experienced in over 150 publications. Thank you most sincerely.”

“The communication between your staff and me has been terrific. Whenever progress is made with the manuscript, I receive notice. Quite honestly, I’ve never had such complete communication with a journal.”

“LA is different, and hopefully represents a kind of scientific publication machinery that removes the hurdles from free flow of scientific thought.”

Your paper will be:

- Available to your entire community free of charge
- Fairly and quickly peer reviewed
- Yours! You retain copyright

<http://www.la-press.com>