Title

Augmented Insulinotropic Action of Arachidonic Acid through the Lipoxygenase Pathway in the Obese Zucker Rat

Journal

Obesity, 8(6)

ISSN

1930-7381

Authors

Ahrén, Bo
Magrum, Linda J
Havel, Peter J
et al.

Publication Date

2000-09-01

DOI

10.1038/oby.2000.59

Peer reviewed

Augmented Insulinotropic Action of Arachidonic Acid through the Lipoxygenase Pathway in the Obese Zucker Rat

Bo Ahrén, Linda J. Magrum,† Peter J. Havel,† Stephanie F. Greene,† Stephen D. Phinney,‡ Patricia R. Johnson,†§ and Judith S. Stern†‡*

Abstract

AHRÉN, BO, LINDA J. MAGRUM, PETER J. HAVEL, STEPHANIE F. GREENE, STEPHEN D. PHINNEY, PATRICIA R. JOHNSON, AND JUDITH S. STERN. Augmented insulinotropic action of arachidonic acid through the lipoxygenase pathway in the obese Zucker rat. *Obes Res.* 2000;8:475–480.

Objective: The metabolism of arachidonic acid (AA) has been shown to be altered in severe insulin resistance that is present in obese (*fa/fa*) Zucker rats. We examined the effects and mechanism of action of AA on basal and glucose-stimulated insulin secretion in pancreatic islets isolated from obese (*fa/fa*) Zucker rats and their homozygous lean (*Fa/Fa*) littermates.

Research Methods and Procedures: Islets were isolated from 10- to 12-week-old rats and incubated for 45 minutes in glucose concentrations ranging from 3.3 to 16.7 mM with or without inhibitors of the cyclooxygenase or lipoxygenase pathways. Medium insulin concentrations were measured by radioimmunoassay, and islet production of the 12-lipoxygenase metabolite, 12-hydroxyeicosatetraenoic acid (12-HETE), was measured by enzyme immunoassay.

Results: In islets from lean animals, AA stimulated insulin secretion at submaximally stimulatory glucose levels (<11.1 mM) but not at 16.7 mM glucose. In contrast, in islets derived from obese rats, AA potentiated insulin secretion at all glucose concentrations. AA-induced insulin secretion was augmented in islets from obese compared with lean rats at high concentrations of AA in the presence of 3.3 mM glucose. Furthermore, the inhibitor of 12-lipoxygenase, esculetin (0.5 μM), inhibited AA-stimulated insulin secretion in islets from obese but not lean rats. Finally, the islet production of the 12-HETE was markedly enhanced in islets from obese rats, both in response to 16.7 mM glucose and to AA.

Discussion: The insulin secretory response to AA is augmented in islets from obese Zucker rats by a mechanism related to enhanced activity of the 12-lipoxygenase pathway. Therefore, augmented action of AA may be a mechanism underlying the adaptation of insulin secretion to the increased demand caused by insulin resistance in these animals.

Key words: Insulin secretion, arachidonic acid, Zucker rats, lipoxygenase

Introduction

In obesity, insulin sensitivity in peripheral tissues is reduced. This reduction is compensated by increased insulin secretion from pancreatic islets, leading to hyperinsulinemia (1–3). If the increase in insulin secretion is inadequate in relation to the demand induced by insulin resistance, glucose intolerance or diabetes evolves (3,4). Although there is evidence for roles by modest hyperglycemia, increased cholinergic activity (5,6), or increased circulating levels of free fatty acids (7) to contribute to the adaptive increase in insulin secretion in insulin resistance, the underlying mechanisms remain incompletely understood. Therefore, investigation of the mechanisms that mediate augmented insulin secretion in insulin resistance is likely to lead to a better understanding of the pathogenesis of glucose intolerance and diabetes. One model useful for the investigation of insulin secretion and action in obesity is the obese (*fa/fa*) Zucker rat, which exhibits hyperphaghia and obesity resulting from a $Gln^{269} \rightarrow Pro$ mutation in the extracellular domain of the leptin receptor (8,9). The net result of the leptin insensitivity is a phenotype characterized by periph-

Submitted for publication October 18, 1999.
Accepted for publication in final form February 24, 2000.
*Department of Medicine, Lund University, Malmö, Sweden and the †Department of Nutrition, ‡Department of Internal Medicine, and §Section of Neurobiology, Physiology and Behavior, University of California, Davis, California.
Address correspondence to Dr. Bo Ahrén, Department of Medicine, Malmö University Hospital, S-205 02 Malmö, Sweden. E-mail: bo.ahren@medforsk.mas.lu.se

eral insulin insensitivity, hyperinsulinemia, and hypersecretion of insulin in response to glucose from enlarged islets (10–15). It has also been shown that in this model the hyperinsulinemia may even precede the peripheral insulin insensitivity (16), making the pathways responsible for insulin secretion in this model of particular interest. Because the fatty Zucker rats do not exhibit overt hyperglycemia and diabetes, this model allows the study of islets from obese, hyperinsulinemic, insulin-resistant animals that have not been chronically exposed to high glucose levels.

Phinney and colleagues (17,18) have established that a relationship exists between the metabolism of arachidonic acid (AA) and obesity in the Zucker rat, including altered distribution of AA in liver and in muscle, and that these abnormalities are correlated with the hyperinsulinemia. In view of these findings, it is possible that altered metabolism of AA or altered responsiveness to AA might contribute to some of the metabolic abnormalities observed in obese Zucker rats. This is of particular relevance for the augmented insulin secretion, because AA is known to function as a second messenger in the signaling pathways regulating insulin secretion through the activation of phospholipase A_2 and diacylglyceride lipase (19–25). AA may also be of importance for insulin secretion by serving as the substrate for several key signaling substances modulating insulin secretion, including those derived from the cyclooxygenase, lipoxygenase, and cytochrome P450 pathways (26–28). It is therefore possible that alterations in AA metabolism contribute to the exaggerated insulin secretion in obesity. In the present study, we have examined the influence of AA on glucose-stimulated insulin secretion from isolated islets derived from obese (*fa/fa*) Zucker rats and their lean littermates (*Fa/Fa*). We have also examined the influence of the inhibitor of cyclooxygenase, sodium salicylate, and the inhibitor of 12-lipoxygenase, esculetin (23,29), on AA-stimulated insulin secretion as well as the production of the 12-lipoxygenase-derived product, 12-hydroxyeicosatetraenoic acid (12-HETE), in the islets.

Research Methods and Procedures

Experimental Animals

Ten- to twelve-week-old male obese (*fa/fa*) and homozygous lean (*Fa/Fa*) Zucker rats were obtained from the Animal Model Core of the Clinical Nutrition Research Unit at the University of California, Davis. Animals were housed in stainless steel, wire-bottom, hanging cages and maintained on a 12-hour light:dark cycle with ad libitum access to food (Purina rat chow) and water.

Isolation of Pancreatic Islets

Pancreatic islets were isolated by the collagenase isolation technique according to Lacy and Kostianovsky (30) with slight modifications. The common bile duct was ligated at the distal end, and the pancreas was distended with 8 mL of Hanks' balanced salt solution (HBSS) (JRH Biosciences). The pancreas was excised, cleaned of fat and other tissue, and minced. Excess HBSS was removed and 6 mL of solution of collagenase, type IX (0.85 mg/ml of 1650 units/mg; Sigma Chemical, St Louis, MO), in HBSS was added. Tissue was digested by gentle shaking in a 37 °C water bath for 9–10 minutes. The digest was rinsed twice with 45 to 50 mL of medium (RPMI 1640 with the addition of 3.3 mM glucose and 10% fetal bovine serum; JRH Biosciences) and resuspended in 30 mL of the same medium. Islets were collected with an Eppendorf pipette under a dissection microscope. Islets were incubated in the above medium for 2 hours at 37 °C in an atmosphere of 5% CO_2 before use in experiments.

Insulin Secretion Experiments

Incubated islets were rinsed with warm Krebs Ringer bicarbonate (KRB) supplemented with 3.3 mM glucose, 5 mM HEPES, pH 7.4, and then transferred (10 islets per well) to 24-well Falcon cell culture plates containing KRB as above. KRB was removed under magnification and replaced with KRB supplemented with glucose, AA (prepared in a stock solution of 500 μM in KRB with 3.3 mM glucose), esculetin, and/or sodium salicylate (all from Sigma) according to the experimental protocols. Islets were then incubated in a shaking water bath at 37 °C in an atmosphere of 95% O_2 and 5% CO_2 for 45 minutes. After incubation, plates were placed on ice, and 0.5 mL of ice-cold KRB supplemented with 0.2% fatty acid free bovine serum albumin (Sigma) was added, resulting in a final concentration of 0.1% bovine serum albumin. The medium was then removed under magnification and stored at −20 °C until it was assayed for insulin or 12-HETE.

Assays

Insulin in the medium was analyzed by radioimmunoassay using a single antibody method and polyethylene precipitation as described previously (31) with the use of rat insulin standard (Novo Biolabs, Wilton, CT), porcine anti-rat insulin (ICN Diagnostics Division, Costa Mesa, CA), ^{125}I-labeled rat insulin (Amersham Pharmacia Biotech, Arlington Heights, IL), and polyethylene glycol (Sigma). 12-HETE was measured by enzyme immunoassay using a kit available from Advanced Magnetics, Inc. (Cambridge, MA).

Statistics

Results are reported as mean ± SEM. For the statistical comparison of means between groups, ANOVA with the Student-Newman-Keul post hoc test and Bonferroni correction for multiple comparison was applied. A p value of ≤ 0.05 was considered statistically significant.

Results

Insulin Secretory Response to AA

The relationship between the media concentrations of AA and insulin secretion at 3.3 mM glucose in islets isolated from lean and obese rats is shown in Figure 1. The insulinotropic action of AA was more pronounced in islets derived from obese animals than from lean animals, as judged by the larger increase in insulin secretion at 250 or 500 μM of AA ($p < 0.05$). Similarly, when islets derived from lean rats were incubated in the presence of AA (100 μM) at glucose concentrations ranging from 3.3 to 16.7 mM, glucose-stimulated insulin secretion was augmented at glucose concentrations lower than 16.7 mM, but not at 16.7 mM glucose ($p < 0.05$ or less). In contrast, in islets from obese animals, AA augmented insulin release at all glucose levels ($p < 0.05$ or less; Figure 2). Thus, at high glucose and at high concentrations of AA, islets from obese animals responded with exaggerated insulin secretion compared with islets from lean rats. In the presence of 16.7 mM glucose, the inhibitor of cyclooxygenase, sodium salicylate (100 μM), stimulated insulin secretion when added together with AA in islets derived from lean rats ($p < 0.05$) but had no effect on AA-stimulated insulin secretion from obese rats. In contrast, the inhibitor of 12-lipoxygenase, esculetin (0.5 μM), inhibited AA-stimulated insulin secretion in islets from obese ($p < 0.01$) but not from lean animals (Figure 3). Together these results suggest that AA-induced insulin secretion from islets obtained from obese animals is less sensitive to inhibition by cyclooxygenase-derived metabolites but more sensitive to inhibition of 12-lipoxygenase than islets from lean animals.

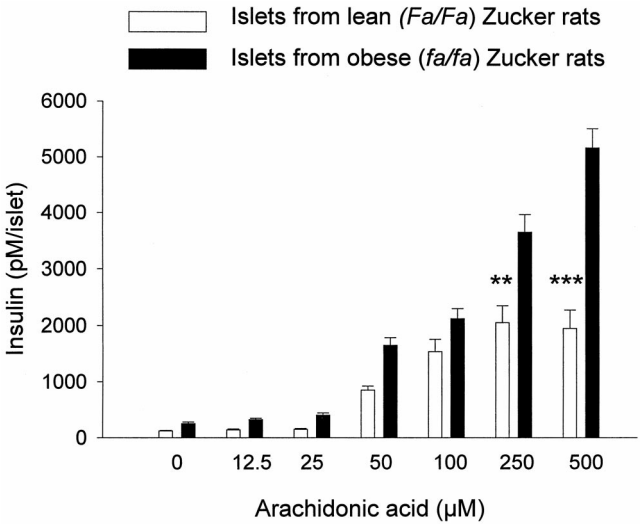

Figure 1. Insulin secretion from islets derived from lean (*Fa/Fa*) or obese (*fa/fa*) Zucker rats incubated in the presence of 3.3 mM glucose and varying concentrations of arachidonic acid. Means ± SEM of four incubations each consisting of 10 islets in each data point are shown. Asterisks indicate the probability level of random difference between groups of islets incubated with and without arachidonic acid (**$p < 0.05$; ***$p < 0.001$).

Islet 12-HETE Production

At 16.7 mM glucose, 12-HETE production was significantly higher in islets isolated from obese rats than from lean rats ($p < 0.01$). At 100 μM, AA markedly increased the release of 12-HETE into the incubation medium containing islets from obese but not from lean rats (Figure 4). Esculetin (0.5μM) reduced the production of 12-HETE induced by AA in islets from obese rats ($p < 0.05$).

Discussion

It has previously been demonstrated that the effect of exogenous AA on insulin secretion from pancreatic islets from non-obese animals is highly dependent on the ambient glucose level, because a stimulation is usually observed at low glucose levels (32–34), whereas an inhibition or no effect is observed at high glucose concentrations (33,34). The stimulatory action of AA on insulin secretion has been thought to be mediated by AA-induced release of Ca^{2+} from intracellular pools, stimulation of the activity of protein kinase C, and through indirect actions induced by lipoxygenase-derived metabolites (22,27,33,35,36). In contrast, the inhibitory action of AA on insulin secretion, seen at high glucose levels, is most likely due to production of substances that inhibit insulin secretion, such as prostaglandins, which are known to counteract the effect of glucose (26). The net effect of AA might therefore depend on the relative formation of cyclooxygenase- and lipoxygenase-derived metabolites.

In this study we confirm that AA stimulates insulin secretion at submaximally stimulatory but not at maximal glucose levels in islets isolated from normal rats. This is consistent with previously reported studies of islets from other strains of rats (32–34). The major finding in the present study is, however, that in contrast to islets from lean animals, the islets obtained from obese Zucker rats exhibited a stimulatory effect of AA also at high glucose concentrations. We also show that the insulinotropic response to AA at high dose levels in the presence of 3.3 mM glucose was higher in islets isolated from obese than from lean rats. Whether this reflects a more marked insulinotropic efficiency of AA also at lower glucose levels or simply the difference in islet size was not examined. Sodium salicylate did not affect this augmented insulin response in islets from obese rats, but the inhibitor or 12-lipoxygenase, esculetin, did. Although we did not verify that cyclooxygenase activity was reduced by salicylate in our experiments, our results suggest that the stimulatory action of AA on insulin secretion from islets from obese rats is dependent on the formation of 12-lipoxygenase-derived products. Lipoxygenase is activated by glucose and by AA (37,38), and through its

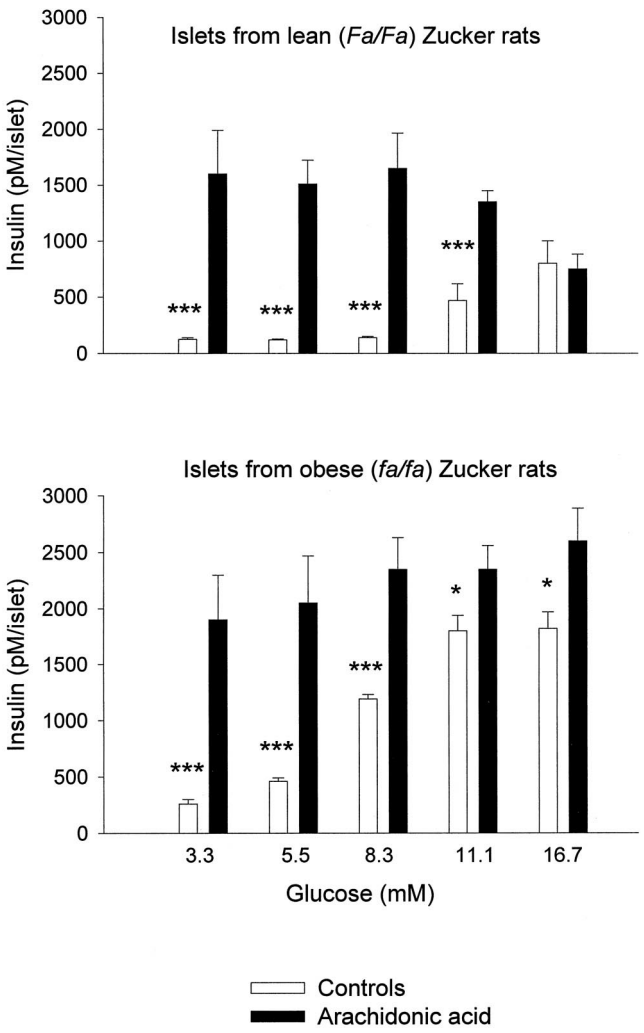

Figure 2. Insulin secretion from islets derived from lean (*Fa/Fa*; upper panel) or obese (*fa/fa*; lower panel) Zucker rats incubated in the presence of 100 μM arachidonic acid and varying concentrations of glucose. Means ± SEM of six incubations each consisting of 10 islets in each data point are shown. Asterisks indicate the probability level of random difference between groups of islets isolated from lean vs. obese Zucker rats (*$p < 0.01$; ***$p < 0.001$).

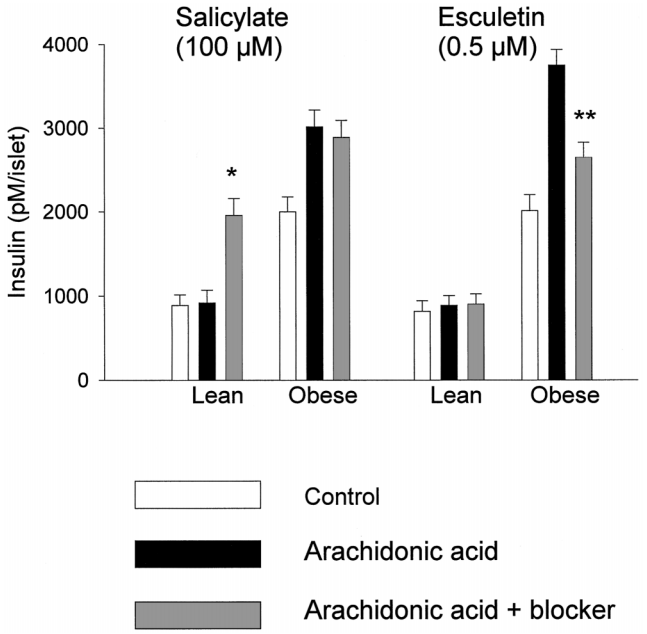

Figure 3. Insulin secretion from islets derived from lean (*Fa/Fa*) or obese (*fa/fa*) Zucker rats incubated in the presence of 16.7 mM glucose, 100 μM arachidonic acid, and sodium salicylate (100 μM) or esculetin (0.5 μM). Means ± SEM of three incubations each consisting of 10 islets in each data point are shown. Asterisks indicate the probability level of random difference between islets incubated with arachidonic acid with or without an inhibitor (*$p < 0.05$; **$p < 0.01$).

activation, 12-HETE, its precursor, 12-hydroperoxyeicosatetraneoic acid (12-HPETE), hepoxilins, and trioxilins are formed (35,37,38). Some of these products have been previously shown to stimulate insulin secretion (27,35,36). Therefore, we examined the production of 12-HETE from islets derived from obese and lean Zucker rats, and we confirmed that 12-HETE is produced by islets (compare with Refs. 37 and 38). We also found that the islet formation of 12-HETE is exaggerated in islets from obese Zucker rats, that esculetin reduced this production, and that the reduction of glucose-stimulated 12-HETE production by esculetin was more pronounced in islets obtained from obese animals than in islets from lean animals. These results suggest that the activity of the 12-lipoxygenase pathway is enhanced in islets from obese rats. Whether this is due to increased expression of or increased substrate availability for the 12-lipoxygenase enzyme remains to be established.

Our results demonstrate that 1) esculetin inhibits the production of 12-HETE and concomitantly inhibits AA-stimulated insulin secretion in islets from obese but not from lean rats and 2) the islets from obese animals exhibit exaggerated production of 12-HETE. Taken together, these results suggest that products of the 12-lipoxygenase pathway are important in the stimulation of insulin secretion by AA in obesity. Esculetin more potently suppressed AA-induced insulin secretion than it reduced 12-HETE formation. This could be due either to a nonlinear relation between these two processes or to other mechanisms of esculetin on insulin secretion that are not related to the lipoxygenase pathway. In any case, the previous demonstration that AA metabolism is altered in obese Zucker rats (5,6), together with the present results, suggests that altered AA metabolism is a significant contributor to the hypersecretion of insulin by the endocrine pancreas of the obese Zucker rat. Whether AA is an important intracellular messenger for islet adaptation to insulin resistance in other models of obesity remains to be studied.

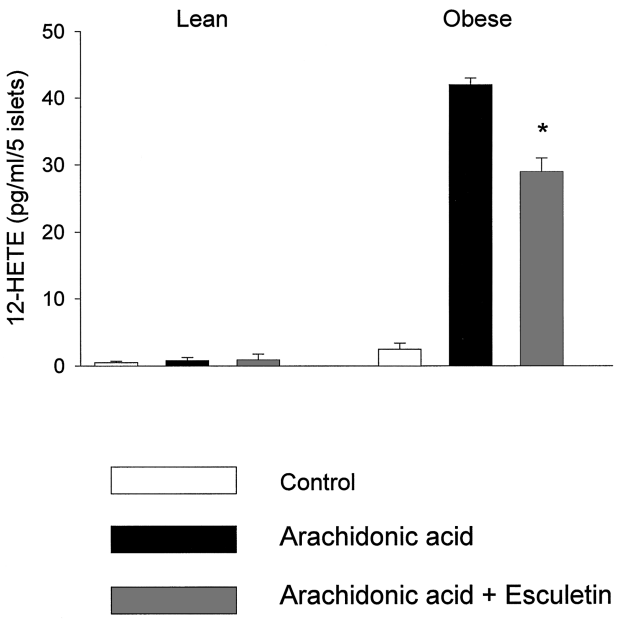

Figure 4. Concentrations of 12-HETE in media from islets derived from lean (*Fa/Fa*) or obese (*fa/fa*) Zucker rats incubated in the presence of 16.7 mM glucose, 100 μM arachidonic acid, and/or esculetin (0.5 μM). Means ± SEM of three incubations each consisting of five islets in each data point are shown. The asterisk indicates the probability level of $p < 0.05$ between islets incubated with arachidonic acid with or without addition of esculetin.

Acknowledgments

The study was supported by a grant from the Nora Eccles Treadwell Foundation; NIH Grants DK-18899, DK-35747, DK-20579, DK-50129, and DK-4073202; Swedish Medical Research Council Grants 14X-6834 and 12X-712; and by the Albert Påhlsson Foundation, the Swedish Diabetes Association, the American Diabetes Association, and the Faculty of Medicine, Lund University.

References

1. **Porte D Jr.** β-Cells in type II diabetes mellitus. *Diabetes.* 1991;40:166–80.
2. **DeFronzo RA, Bonnadonna RC, Ferranini E.** Pathogenesis of NIDDM: a balanced overview. *Diabetes Care.* 1992;15: 318–68.
3. **Larsson H, Ahrén B.** Islet dysfunction in obese women with impaired glucose tolerance. *Metabolism.* 1996;45:502–9.
4. **Larsson H, Ahrén B.** Failure to adequately adapt reduced insulin sensitivity with increased insulin secretion in women with impaired glucose tolerance. *Diabetologia.* 1996;39:1099–107.
5. **Ahrén B, Simonsson E, Scheurink AJW, Mulder H, Myrsén U, Sundler F.** Dissociated insulinotropic sensitivity to glucose and carbachol in high-fat diet-induced insulin resistance in C57BL/6J mice. *Metabolism.* 1997;46:97–106.
6. **Ahrén B, Sauerberg P, Thomsen C.** Increased insulin secretion and normalisation of glucose tolerance by cholinergic agonism in high-fat fed C57BL/6J mice. *Am J Physiol.* 1999; 277:E93–E102.
7. **McGarry JD, Dobbins RL.** Fatty acids, lipotoxicity and insulin secretion. *Diabetologia.* 1999;42:128–38.
8. **Philips MS, Liu QY, Hammond HA, et al.** Leptin receptor missense mutation in the fatty Zucker rat. *Nat Genet.* 1996; 13:18–9.
9. **Iida M, Murakami T, Ishida K, Mizuno A, Kuwajima M, Shima K.** Substitution at codon 269 (glutamine→proline) of the leptin receptor (OB-R) cDNA is the only mutation found in the Zucker fatty (*fa/fa*) rat. *Biochem Biophys Res Commun.* 1996;224:597–604.
10. **Zucker LM, Antoniades H.** Insulin and obesity in the Zucker genetically obese rat "fatty." *Endocrinology.* 1972;90:1320–30.
11. **Stern JS, Johnson PR, Batchelor BR, Zucker LM, Hirsch J.** Pancreatic insulin release and peripheral tissue resistance in Zucker obese rats fed high- and low-carbohydrate diets. *Am J Physiol.* 1975;228:543–8.
12. **Blonz ER, Stern JS, Curry DL.** Dynamics of pancreatic insulin release in young Zucker rats: a heterozygote effect. *Am J Physiol.* 1985;248:E188–E93.
13. **Johnson PR, Stern JS, Greenwood MR, Hirsch J.** Adipose tissue hyperplasia and hyperinsulinemia of Zucker obese female rats: a developmental study. *Metabolism.* 1978;27:1941–54.
14. **Curry DL, Stern JS.** Dynamics of insulin hypersecretion by obese Zucker rats. *Metabolism.* 1985;34:791–6.
15. **Kuffert A, Stern JS, Curry DL.** Pancreatic hypersensitivity to glucose by young obese Zucker rats (*fa/fa*). *Metabolism.* 1988;37:952–7.
16. **Turkenkopf IJ, Johnson PR, Greenwood MRC.** Development of pancreatic and plasma insulin in prenatal and suckling Zucker rats. *Am J Physiol.* 1982;242:E220–5.
17. **Phinney SD, Tang AB, Thurmond DC, Nakamura MT, Stern JS.** Abnormal polyunsaturated lipid metabolism in the obese Zucker rat, with partial metabolic correction by gamma-linolenic acid administration. *Metabolism.* 1992;42:1127–40.
18. **Ayre KJ, Phinney SD, Tang AB, Stern JS.** Exercise training reduces skeletal muscle membrane arachidonate in the obese (*fa/fa*) Zucker rat. *J Appl Physiol.* 1998;85:1898–902.
19. **Jones PM, Persaud SJ.** Arachidonic acid as a second messenger in glucose-induced insulin secretion from pancreatic beta-cells. *J Clin Endocrinol.* 1993;137:7–14.
20. **Loweth AC, Scarpello JB, Morgan NG.** Phospholipase A_2 expression in human and rodent insulin-secreting cells. *Mol Cell Endocrinol.* 1995;112:177–83.
21. **Gross RW, Ramanadham S, Kruszka KK, Han X, Turk J.** Rat and human pancreatic islet cells contain a calcium ion independent phospholipase A_2 activity selective for hydrolysis of arachidonate which is stimulated by adenosine trisphosphate and is specifically localized to islet beta-cells. *Biochemistry.* 1993;32:327–36.
22. **Ramandham S, Gross RW, Han X, Turk J.** Inhibition of arachidonate release suppresses both insulin secretion and the rise in β-cell cytosolic Ca^{2+} concentration. *Biochemistry.* 1993;32:337–46.

23. **Simonsson E, Karlsson S, Ahrén B.** Involvement of phospholipase A_2 and arachidonic acid in cholecystokinin-8-induced insulin secretion. *Regul Pept.* 1996;65:101–7.
24. **Simonsson E, Karlsson S, Ahrén B.** Ca^{2+}-independent phospholipase A_2 contributes to the insulinotropic action of cholecystokinin-8 in rat islets: dissociation from the mechanism of carbachol. *Diabetes.* 1998;47:1436–43.
25. **Konrad RJ, Major CD, Wolf BA.** Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: sequential actions of diacylglycerol and monoacylglycerol lipases. *Biochemistry.* 1994;33:13284–94.
26. **Robertson RP.** Eicosanoids as pluripotential modulators of pancreatic islet function. *Diabetes.* 1988;37:367–70.
27. **Metz SA.** Putative roles for lysophospholipids as mediators and lipoxygenase-mediated metabolites of arachidonic acid as potentiators of stimulus-secretion coupling: dual mechanisms of *p*-hydroxymercuribenzoic acid-induced insulin release. *J Pharmacol Exp Ther.* 1986;238:819–32.
28. **Falck JR, Manna S, Moltz J, Chacos N, Capdevila J.** Epoxyeicosatrienoic acids stimulate glucagon and insulin release from isolated rat pancreatic islets. *Biochem Biophys Res Commun.* 1983;114:743–9.
29. **Huang HC, Lai MW, Wang HR, Chung YL, Hsieh LM, Chen CC.** Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction pathways. *Eur J Pharmacol.* 1993;237:39–44.
30. **Lacy PE, Kostianovsky M.** Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* 1967;16:35–9.
31. **Desbuquois B, Aurbach GD.** Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab.* 1971;33:732–8.
32. **Metz SA.** Exogenous arachidonic acid promotes insulin release from intact or permeabilized rat islets by dual mechanisms: putative activation of Ca^{2+} mobilization and protein kinase C. *Diabetes.* 1988;37:1453–69.
33. **Band AM, Jones PM, Howell SL.** The mechanism of arachidonic acid-induced insulin secretion from rat islets of Langerhans. *J Mol Endocrinol.* 1992;8:95–101.
34. **Landt M, Easom RA, Colca JR, et al.** Parallel effects of arachidonic acid on insulin secretion, calmodulin-dependent protein kinase activity and protein kinase C activity in pancreatic islets. *Cell Calcium.* 1992;13:163–72.
35. **Pace-Asciak CR, Martin JM, Corey EJ, Su WG.** Endogenous release of hepoxilin A3 from isolated perifused pancreatic islets of Langerhans. *Biochem Biophys Res Commun.* 1985;128:942–6.
36. **Metz SA, Fujimoto WY, Robertson RP.** A role for the lipoxygenase pathway of arachidonic acid metabolism in glucose- and glucagon-induced insulin secretion. *Life Sci.* 1983;32:903–10.
37. **Metz SA.** Glucose increases the synthesis of lipoxygenase-mediated metabolites of arachidonic acid in intact rat islets. *Proc Natl Acad Sci USA.* 1985;82:198–202.
38. **Shannon VR, Ramanadham S, Turk J, Holtzman MJ.** Selective expression of an arachidonate 12-lipoxygenase by pancreatic islet beta-cells. *Am J Physiol.* 1992;263:E828–E36.