Development of Insulin Resistance and Endothelin-1 Levels in the Zucker Fatty Rat

Nathalie Berthiaume, Amanda K. Mika, and Bradley A. Zinker

In order to determine the effects of increasing insulin resistance on endothelin-1 (ET-1) levels, Zucker lean and fatty rats were studied at basal and during a complete nutrient meal tolerance test (MTT) at 7, 12, and 15 weeks of age. The fatty rats were mildly hyperglycemic, severely hyperinsulinemic and glucose-intolerant at all ages versus lean animals and this progressed with age within groups, as previously published. Basal ET-1 levels, at 7 weeks, were significantly increased in fatty versus lean rats (3.2 ± 0.5 *v* 2.0 ± 0.3 pg/mL, respectively; *P* < .05); however, we did not observe any significant basal difference at 12 or 15 weeks. At 7 weeks, ET-1 levels between fatty and lean rats were not different during the MTT (15 minutes: 2.9 ± 0.4 *v* 2.7 ± 0.7; 120 minutes: 6.5 ± 0.8 *v* 6.6 ± 0.5 pg/mL, fatty *v* lean, respectively). At 12 weeks, though there was no difference in basal levels, fatty rats had higher ET-1 levels during the MTT compared to lean animals (15 minutes: 6.9 ± 1.4 *v* 1.8 ± 0.4; 120 minutes: 9.4 ± 1.7 *v* 3.2 ± 0.5 pg/mL, respectively; *P* < .01). At 15 weeks, ET-1 levels during the MTT receded to levels similar to those observed at 7 weeks, which were significantly higher in fatty versus lean rats 15 minutes following the challenge (3.4 ± 0.4 *v* 2.4 ± 0.2 pg/mL, respectively; *P* < .05). In conclusion, ET-1 levels in the Zucker fatty rat: (1) were increased in the early stages of the progression of insulin resistance at 7 weeks, but were unchanged under basal conditions with age thereafter, and (2) were increased under nutrient challenge conditions with advanced insulin resistance up to 12 weeks, and were still significantly but to a lesser degree increased at 15 weeks of age. The explanation for these results and their relationship to the observed insulin resistance is unclear and will require further investigation.

THE GENETICALLY OBESE Zucker fatty rat (*fa/fa)* is a commonly used model of insulin resistance characterized by mild hyperglycemia, abnormal oral glucose tolerance, hyperinsulinemia, and insulin resistance of the liver, skeletal muscle, and fat tissues.[1-3] A mutation of the leptin receptor gene is responsible for the diseased state in this animal model.[4,5]

Endothelin (ET) is an autacoid discovered from the supernatant of porcine aortic endothelial cells.[6] It is still the most potent vasoconstrictor peptide known.[6] In addition to endothelin's well-known effects on the cardiovascular system,[7] this peptide could be involved in pathologies such as insulin resistance and diabetes and their complications.[8,9]

Increased circulating ET-1 levels have been reported during a euglycemic-hyperinsulinemic clamp in lean non–insulin-dependent diabetes mellitus (NIDDM) men,[10] as well as in obese patients with the metabolic syndrome,[11] when compared to normal subjects. Although basal ET-1 levels were shown to be similar, it has been reported that plasma ET-1 and insulin levels, following a glucose load, were significantly higher in non-obese nondiabetic essential hypertensive subjects compared to normotensive subjects.[12] Consistent with these observations, it has been demonstrated that hypertriglyceridemia and/or hyperinsulinemia are potent inducers of ET-1 release in humans with syndrome X, with insulinoma, as well as in normal subjects.[13] On the contrary, Gregersen et al demonstrated in vitro that ET-1 stimulates insulin secretion by direct action on mice isolated islets of Langerhans.[14] The same group showed that ET-1 has also an insulinotropic effect in rat pancreatic alpha cells, secondary to the stimulation of glucagon release.[15] It appears that, as damaged endothelium is a consequence of insulin resistance and/or diabetes,[15] leakage from damaged blood vessels would increase ET-1 release. Increased ET-1 levels would, in turn, increase glucagon secretion from the pancreas and, subsequently, insulin release.

Recently, several studies have demonstrated that combined use of insulin and ET-1 causes decreased insulin-stimulated glucose uptake in rat adipocytes in vitro.[16-18] Two other studies, however, showed the opposite effect, ie, endothelin stimulates glucose uptake and GLUT4 translocation in 3T3-L1 adipocytes.[19,20] Possible explanations for this discrepancy are the differences in the time of exposure to ET-1 (~2 hours *v* 30 minutes)[16-18,20] and/or the type of cells used (rat isolated adipocytes *v* 3T3-L1).[16-18,19-20] In fact, it has also been observed that longer exposure to ET-1 (6 to 24 hours) leads to the desensitization of the insulin signaling pathway and decreases glucose uptake in 3T3-L1 adipocytes.[21]

From these results the role of ET-1 in the pathogenesis of insulin resistance and/or diabetes remains unclear. The purpose of the current investigation was to characterize the effects of age, the progression of insulin resistance, and the stimulus of a nutrient challenge, on plasma ET-1 levels, in a commonly used model of insulin resistance, the Zucker fatty rat in vivo.

MATERIALS AND METHODS

Animals Used

Animals were treated in conformity with the Abbott Laboratories institutional animal care and use committee (IACUC) guidelines.

Eighteen to twenty 6- to 7-week-old genetically obese Zucker fatty (*fa/fa*) rats and 18 to 20 lean rats obtained from Harlan (Madison, WI) were used for the experiments described below. Rats were housed in

From the Global Pharmaceutical Research and Development Division, Metabolic Disease Research, Abbott Laboratories, Abbott Park, IL.

Submitted August 16, 2002; accepted February 8, 2003.

Supported by a post-doctoral Fellowship from the University-Industry Partnership program of the Canadian Institutes of Health Research (CIHR) and Abbott Laboratories.

Address reprint requests to Bradley A. Zinker, PhD, Department R47M, Bldg AP13A, 2nd Floor, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064-3500.

0026-0495/03/5207-0010$30.00/0

doi:10.1016/S0026-0495(03)00098-2

standard animal cages (4 per cage) on a 12-hour light/12-hour dark cycle and given free access to water and rat chow (Teklad rodent diet 8640, Harlan). They were acclimatized for 1 week before any study was started. This longitudinal study was repeated 2 times with 9 or 10 Zucker fatty and 9 or 10 lean rats of the same age and from the same vendor. Since the results were not statistically different between each study, results from both studies were pooled and, therefore, there were 18 to 20 animals per group.

Meal Tolerance Test-Complete Nutrient Challenge

A meal tolerance test (MTT) was performed in conscious 7- to 8-week old rats following an overnight fast (at ~8 AM) as previously described by our group.[22] The MTT consisted of a complete nutrient challenge (Ensure Plus, Abbott Laboratories, Ross Products Division, Columbus, OH) and was administered by oral gavage at a dose of 1.2 g carbohydrate/kg body weight. Blood samples were obtained following the challenge for determination of plasma glucose and insulin (~50-μL samples) (0, 15, 30, 60, and 120 minutes) and ET-1 (~500-μL samples) (0, 15, and 120 minutes) levels. The same rats were used to perform the subsequent MTTs and determine glucose, insulin, and ET-1 levels at 12 and 15 weeks of age.

Glucose and Insulin Determinations

Plasma glucose was determined immediately from fresh samples with the Medisense Precision G blood glucose testing system (Medisense Products, Abbott Laboratories, Bedford, MA). Plasma insulin was measured by a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Alpco Diagnostics, Winham, NH). Rat insulin standard was used. The coefficient of variation (C.V.) (interassay) was approximately 4%.

Incremental area under the plasma glucose or insulin response curves (AUC) was calculated according to Wolever and Jenkins.[23]

ET-1 Measurement

Plasma ET-1 levels were determined with the QuantiGlo human ET-1 chemiluminescent immunoassay (R & D Systems, Minneapolis, MN). The first incubation step was performed at 4°C, overnight, instead of 1.5 hours at room temperature, in order to improve the sensitivity of the assay. In addition, dilutions of 0.8, 4, and 20 pg/mL were included into the standard curve to widen the range of concentrations.

Statistical Analysis

Results are given as mean ± SEM for the indicated number of rats. A 1-way analysis of variance (ANOVA) with repeated measures followed by a Tukey-Kramer multiple comparisons test was used. P values ≤ .05 (2-tailed) were considered significant.

RESULTS

Body weight increased with age in both Zucker lean and fatty (*fa/fa*) rats as anticipated. In addition, body weights were significantly higher in Zucker fatty rats compared to lean animals, at all ages (data not shown).

Overnight fasted plasma glucose levels (0 minutes) were significantly higher, at all ages, in Zucker fatty rats compared to their lean counterparts, as anticipated (Fig 1A and B) (n = 18 to 20 per group, $P < .01$). At 7 weeks, glucose levels peaked 15 minutes following the onset of the MTT in both lean and fatty animals (Fig 1 A and B). However, these levels peaked 60 minutes following the onset of the MTT, at 12 and 15 weeks (Fig 1A and B). Zucker fatty rats were significantly glucose intolerant, in response to the MTT, compared to the lean

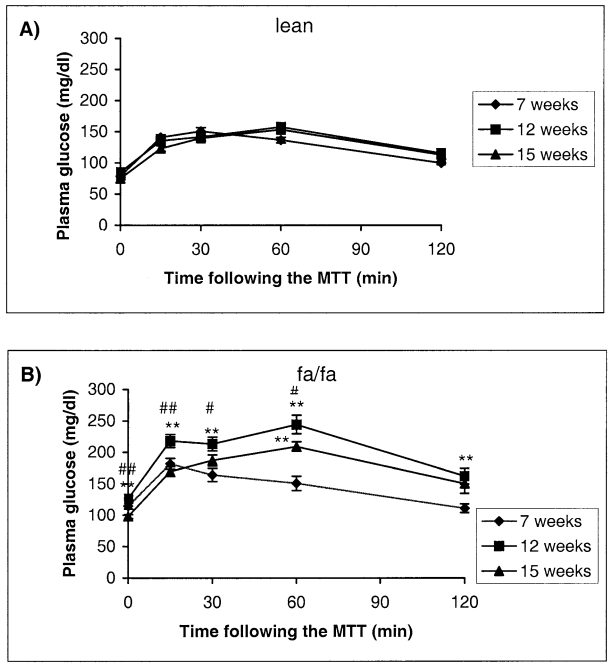

Fig 1. Plasma glucose levels before and following a MTT in Zucker lean (A) and fatty rats (B) at 7, 12, and 15 weeks of age. Each symbol with a bar represents the mean ± SEM of 18 to 20 animals per group. **$P < .01$ *v* 7 weeks; #$P < .05$, 12 *v* 15 weeks; ##$P < .01$, 12 *v* 15 weeks.

animals, at 12 and 15 weeks but not at 7 weeks (Fig 1A and B). However, there was no further progression of glucose intolerance between 12 and 15 weeks of age for the fatty animals (Fig 1B). This is also illustrated by the incremental glucose AUC ($P < .01$, 7 *v* 12 weeks; not significantly different, 12 *v* 15 weeks). It should be noted that the incremental glucose AUC for the fatty animals is slightly, although significantly, lower compared to lean animals at 7 weeks. However, the peak glucose values were significantly higher in the fatty rats at 7 weeks (181.8 ± 8.5 *v* 150.8 ± 5.8 mg/dL; $P < .01$, Zucker fatty *v* lean rats, respectively).

Overnight fasted plasma insulin levels were significantly higher, at all ages, in fatty rats compared to their lean littermates, as expected in this model (Fig 2A and B). In addition, the fatty animals became more hyperinsulinemic with age as illustrated by the overnight fasted (basal) levels ($P < .01$) (Fig 2B). Nevertheless, there was no further progression of the hyperinsulinemia in the fatty animals between 12 and 15 weeks (Fig 2B). Insulin levels peaked 15 minutes following the meal challenge, at all ages, and were significantly higher in fatty compared to lean rats ($P < .01$)(Fig 2A and B). Finally, the incremental insulin AUC significantly increased with age in both lean and fatty animals (Fig 3B). Incremental insulin AUC values were also significantly elevated, at all ages, in fatty animals compared to their lean littermates ($P < .01$) (Fig 3B).

Alhough basal ET-1 levels, at 7 weeks, were minimally but significantly increased in fatty *v* lean rats ($P < .05$) (Fig 4A), they were not significantly different between the 2 groups at 12 and 15 weeks ($P = .08$). Plasma ET-1 levels were significantly higher at 12 weeks in fatty animals compared to their lean

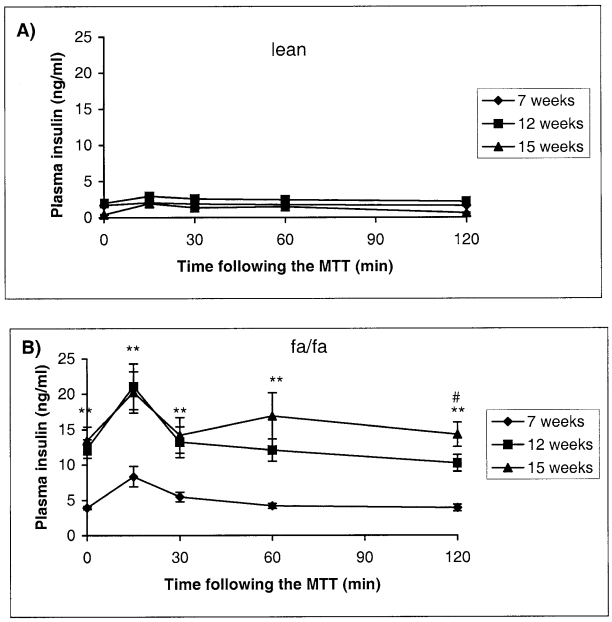

Fig 2. Plasma insulin levels before and following a MTT in Zucker lean (A) and fatty rats (B) at 7, 12, and 15 weeks of age. Each symbol with a bar represents the mean ± SEM of 18 to 20 animals per group. **$P < .01$, *v* 7 weeks; #$P < .05$, 12 *v* 15 weeks.

counterparts, 15 and 120 minutes following the MTT ($P < .01$) (Fig 4B). This represents a 4-fold and a 3-fold increase in ET-1 levels at 15 and 120 minutes, respectively. It is also noteworthy that ET-1 levels in fatty rats were significantly higher at 12 weeks compared to 7 or 15 weeks, 15 and 120 minutes following the challenge ($P < .01$) (Fig 4A through C). Finally, at 15 weeks of age, plasma ET-1 levels receded back to similar levels observed at 7 weeks. However, these levels were still significantly higher in Zucker fatty rats compared to their lean counterparts 15 minutes following the MTT ($P < .05$) (Fig 4C).

DISCUSSION

In this study, we monitored plasma ET-1 levels in glucose intolerant, hyperinsulinemic, insulin-resistant rats during an 8-week growth period (from 7 to 15 weeks of age) and under nutrient-challenged conditions. To our knowledge, this is the first investigation describing the effects of age, the progression of insulin resistance, and the effects of a complete nutrient challenge, on plasma ET-1 levels in the commonly used model of insulin resistance, the Zucker fatty rat. In addition, lean littermates were studied for comparison purposes.

In agreement with previous investigations,[1-3] we observed that Zucker fatty rats were mildly hyperglycemic after an overnight fast and significantly glucose-intolerant compared to their lean counterparts. In addition, fatty animals became progressively more glucose-intolerant with age, as anticipated.[1-3]

Our glucose excursion results are also in accordance with previously published work conducted in the same animal model, but using an oral glucose tolerance test (OGTT) instead of a MTT.[1] We chose a complete nutrient MTT compared to the commonly used OGTT to characterize the metabolic responses and ET-1 levels, since we have previously demonstrated that a MTT stimulates a greater combination of glucose and insulin elevations in comparison with an OGTT in the same animal model.[22] It should be mentioned that even though the glucose AUC in the Zucker fatty rat at 7 weeks was significantly lower compared to its lean counterparts, the baseline and peak glucose responses were significantly higher in the fatty rats compared to their lean littermates. This suggests the expected development of glucose intolerance in this model.

The Zucker fatty rats were hyperinsulinemic, at all ages, compared to their lean littermates, as anticipated.[1-3] In addition, the insulin resistance progressed with age and was significantly increased in fatty animals compared to their lean littermates, as illustrated by the baseline and peak insulin responses as well as by the incremental insulin AUC. We also detected a significant rise in the insulin AUC with age in the lean littermates, indicating a progression of insulin resistance in these rats as well. However, this was not to the same extent as that observed in the fatty animals.

We observed a significant increase in basal ET-1 levels, at 7 weeks of age, in fatty rats compared to their lean counterparts. However, we did not detect any significant difference at 12 and 15 weeks. The physiological meaningfulness of this result is not clear. Nevertheless, our observations on basal ET-1 levels are in agreement with a previous investigation showing no

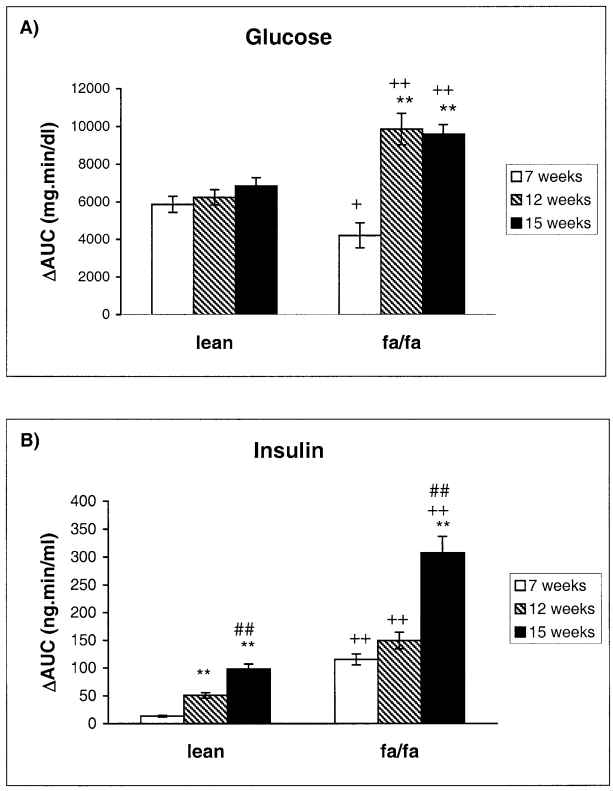

Fig 3. (A) Glucose and (B) insulin AUC in Zucker lean and fatty rats at 7, 12, and 15 weeks of age. Each column with a bar represents the mean ± SEM of 18 to 20 animals per group. **$P < .01$ *v* 7 weeks; ##$P < .01$, 12 *v* 15 weeks; +$P < .05$, lean *v* fatty; ++$P < .01$, lean *v* fatty (respective to age).

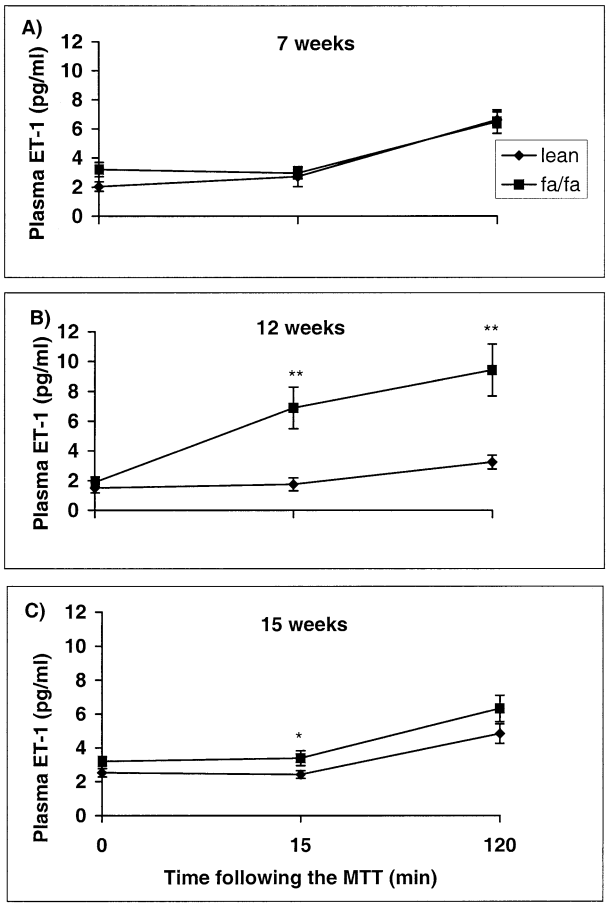

Fig 4. Plasma ET-1 levels before, 15 minutes, or 120 minutes following a MTT in (A) 7-week-old, (B) 12-week-old, or (C) 15-week-old Zucker lean and fatty rats. Each symbol with a bar represents the mean ± SEM of 18 to 20 animals per group. *$P < .05$ and **$P < .01$, lean *v* fatty.

significant difference in ET-1 levels between Zucker lean and fatty rats at 12 and 40 weeks of age.[24] The effects of a nutrient or glucose challenge, as well as possible differences in younger lean and fatty rats, were not investigated in this earlier study.[24] Our results are also consistent with human studies reporting elevated ET-1 levels in people with diabetes under euglycemic-hyperinsulinemic conditions,[10] nondiabetic hypertensive individuals under glucose load conditions,[12] and subjects with metabolic syndrome,[11] compared to control individuals.

Plasma ET-1 levels, at all ages, increased in both lean and fatty rats during the meal challenge. In addition, plasma ET-1 levels were significantly increased in Zucker fatty rats compared to their lean littermates, 15 and 120 minutes following a meal challenge, at 12 weeks of age but not at 7 weeks. This result at 12 weeks is consistent with the acute human study reported by Ferri et al.[12] Although these investigators did not monitor their subjects over time (age), they observed significantly increased plasma glucose, insulin, and ET-1 levels following a glucose load in nondiabetic essential hypertensive compared to normotensive subjects.[12] It is noteworthy that basal glucose, insulin, and ET-1 levels were similar in both groups of human subjects. Interestingly, an acute human study also reported elevated ET-1 levels in type 2 diabetic patients compared to nondiabetic controls, 60 and 120 minutes during a euglycemic-hyperinsulinemic clamp.[10] These levels were no longer elevated 150 minutes following the clamp.[10]

One possible explanation for the ET-1 results observed at 12 weeks during the meal challenge is that the meal challenge itself increases insulin release from the pancreas, which then lead to the more pronounced ET-1 elevation. Since it has been demonstrated that insulin is a potent inducer of ET-1 release,[13] the increased insulin release following the MTT in the Zucker fatty rat could have triggered the greater increase in ET-1 secretion in comparison to its lean counterpart. However, the reported results[13]do not explain the lack of difference in basal ET-1 levels observed between fatty and lean animals at 12 and 15 weeks of age.

Finally, with advancing age, we observed that ET-1 levels during the MTT at 15 weeks receded back to similar levels observed at 7 weeks. However, plasma ET-1 levels, following the meal challenge, were still significantly higher at 15 minutes in the fatty animals compared to their lean littermates, although not as dramatically as at 12 weeks. One possible explanation for this observation could be an increased degradation rate of ET-1 with age. A more plausible explanation, however, would be that with increasing hyperinsulinemia and the progression of insulin resistance, the animals loose their capacity to stimulate ET-1 secretion or present an attenuated ET-1 production with age. This is supported by the decreased preproET-1 (ET-1 precursor) mRNA levels reported in isolated aorta from Zucker fatty rats compared to their lean littermates.[24] Furthermore, it could be that there are some adaptative mechanisms that occur with increased age that have thus far not been determined.

In conclusion, the present results describe the progression of insulin resistance in relation to plasma ET-1 levels under basal and nutrient-challenged conditions in the Zucker fatty rat in comparison to their lean counterparts. ET-1 levels in the Zucker fatty rat: (1) were increased in the early stages of the progression of insulin resistance at 7 weeks of age, but were unchanged under basal conditions with age thereafter, and (2) were increased under nutrient challenge conditions with advanced insulin resistance up to 12 weeks of age, and were still significantly but to a lesser degree increased at 15 weeks. Furthermore, the reduction in the rise in ET-1 levels with age, at 15 weeks, during a complete nutrient challenge may provide insights into the pathogenesis of insulin resistance that could also be investigated. The mechanisms involved, the full impact, and the exact role of ET-1 in insulin resistance will require further investigation.

REFERENCES

1. Apweiler R, Freund P: Development of glucose intolerance in obese (*fa/fa*) Zucker rats. Horm Metab Res 25:521-524, 1993

2. Ionescu E, Sauter JF, Jeanrenaud B: Abnormal glucose tolerance in genetically obese (*fa/fa*) rats. Am J Physiol 248:E500-E506, 1985

3. Terretaz J, Assimacopoulos-Jeannet F, Jeanrenaud B: Severe

hepatic and peripheral insulin resistance as evidenced by euglycemic clamps in genetically obese *fa/fa* rats. Endocrinology 118:674-678, 1986

4. Phillips MS, Liu Q, Hammond HA, et al: Leptin receptor missense mutation in the fatty Zucker rat. Nat Genet 13:18-19, 1996

5. Tartaglia LA: The leptin receptor. J Biol Chem 272:6093-6096, 1997

6. Yanagisawa M, Kurihara S, Kimura Y, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411-415, 1988

7. Miyauchi T, Masaki T: Pathophysiology of endothelin in the cardiovascular system. Ann Rev Physiol 61:391-415, 1999

8. Rubanyi GM, Polokoff MA: Endothelins: Molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol Rev 46:325-415, 1994

9. Lam HC: Role of endothelin in diabetic vascular complications. Endocrine 14:277-284, 2001

10. Ferri C, Carlomagno A, Coassin S, et al: Circulating endothelin-1 levels increase during euglycemic hyperinsulinemic clamp in lean NIDDM men. Diabetes Care 18:226-233, 1995

11. Ferri C, Bellini C, Desideri G, et al: Circulating endothelin-1 levels in obese patients with the metabolic syndrome. Exp Clin Endocrinol Diabetes 105:38-40, 1997 (suppl 2)

12. Ferri C, Bellini C, Desideri G, et al: Endogenous insulin modulates circulating endothelin-1 concentrations in humans. Diabetes Care 19:504-506, 1996

13. Piatti P, Monti LD, Conti M, et al: Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes 45:316-321, 1996

14. Gregersen S, Thomsen JL, Brock B, et al: Endothelin-1 stimulates insulin secretion by direct action on the islets of Langerhans in mice. Diabetologia 39:1030-1035, 1996

15. Brock B, Gregersen S, Kristensen K, et al: The insulinotropic effect of endothelin-1 is mediated by glucagon release from the islet alpha cells. Diabetologia 42:1302-1307, 1999

16. Chou YC, Perng JC, Juan CC, et al: Endothelin-1 inhibits insulin-stimulated glucose uptake in isolated rat adipocytes. Biochem Biophys Res Commun 202:688-693, 1994

17. Lee YC, Juan CC, Fang VS, et al: Evidence that endothelin-1 inhibits insulin-stimulated glucose uptake in rat adipocytes mainly through ET_A receptors. Metabolism 47:1468-1471, 1998

18. Shih KC, Kwok CF, Ho LT: Combined use of insulin and endothelin-1 causes decrease of protein expression of β-subunit of insulin receptor, insulin receptor substrate-1 and insulin stimulated glucose uptake in rat adipocytes. J Cell Biochem 78:231-240, 2000

19. Ishibashi K, Imamura T, Sharma PM, et al: The acute and chronic stimulatory effects of endothelin-1 on glucose transport are mediated by distinct pathways in 3T3-L1 adipocytes. Endocrinology 141:4623-4628, 2000

20. Wu-Wong JR, Berg CE, Wang J, et al: Endothelin stimulated glucose uptake and Glut4 translocation via activation of endothelin ETA receptor in 3T3-L1 adipocytes. J Biol Chem 274:8103-8110, 1999

21. Ishibashi K, Imamura T, Sharma PM, et al: Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3T3-L1 adipocytes. J Clin Invest 107:1193-1202, 2001

22. Berthiaume N, Zinker B: Metabolic responses in a model of insulin resistance: Comparison between oral glucose and meal tolerance tests. Metabolism 5:595-598, 2002

23. Wolever TMS, Jenkins JA: The use of the glycemic index in predicting the blood glucose response to mixed meals. Am J Clin Nutr 43:167-172, 1986

24. Wu SQ, Hopfner RL, McNeill JR, et al: Altered paracrine effect of endothelin in blood vessels of the hyperinsulinemic, insulin resistant obese Zucker rat. Cardiovasc Res 45:994-1000, 2000