Longitudinal Study of Carbohydrate Metabolism in Healthy Obese Pregnant Women

EYAL SIVAN, MD
XINHUA CHEN, MD
CAROL J. HOMKO, RN, MS
E. ALBERT REECE, MD
GUENTHER BODEN, MD

OBJECTIVE — To longitudinally characterize changes in insulin sensitivity in obese women during and after pregnancy.

RESEARCH DESIGN AND METHODS — Six glucose-tolerant obese women underwent a 4-h euglycemic-hyperinsulinemic (500–600 pmol/l) clamping during the second (22.5 ± 2 weeks [mean ± SD]) and third trimester (36.8 ± 0.9) of pregnancy and again 15.6 ± 1.4 weeks after delivery. Rates of total body glucose turnover (with [6,6-$^2H_2$]glucose) and oxidation (with indirect calorimetry) were measured.

RESULTS — There were no significant changes with respect to the action of insulin on rates of glucose disappearance (G_{Rd}), carbohydrate oxidation, or endogenous glucose production (EGP), comparing the second trimester of pregnancy with the nonpregnant (postpartum) state. The third trimester, however, was characterized *1*) by reductions in insulin-stimulated G_{Rd} (−28%, $P < 0.05$, compared with the second trimester and −40%, $P < 0.05$, compared with postpartum); 2) by even larger reductions in insulin-stimulated carbohydrate oxidation (−46%, $P < 0.05$, compared with the second trimester and −54%, $P < 0.02$, compared with postpartum); and 3) by reduction of insulin suppression of EGP (−39% compared with −79% at the second trimester and −77% postpartum, $P < 0.01$).

CONCLUSIONS — Glucose-tolerant obese women developed peripheral as well as hepatic insulin resistance during the third trimester of pregnancy. These alterations were reversed after delivery and appeared to be adaptive mechanisms to cope with the increased demand for glucose of the growing fetus.

Insulin resistance during pregnancy is a well-recognized phenomenon (1–3). Changes in insulin sensitivity during pregnancy have been examined by a variety of techniques. Semiquantitative information was obtained by calculating insulin to glucose ratios (4). More recently, quantitative techniques including hyperinsulinemic-euglycemic clamping (5–7) and minimal model analysis of intravenous glucose tolerance tests (8) have been used in cross-sectional studies comparing late gestation to the postpartum period. Catalano et al. (6) were the first to longitudinally assess changes in insulin sensitivity during gestation using the hyperinsulinemic-euglycemic clamp technique. They demonstrated an ~50% reduction in peripheral insulin sensitivity from early-to-late gestation in normal-weight pregnant women. Similar studies, however, have not been done in obese pregnant women. This information could be important because obesity is known to be associated with an increase in insulin resistance (9) and because obese women are at a greater risk for the development of both gestational diabetes (10,11) and NIDDM (12,13); hence, insulin resistance is likely to be more severe in obese than in lean pregnant women. The purpose of the current study was, therefore, to characterize changes in insulin sensitivity in obese women throughout pregnancy and after delivery.

From the Departments of Obstetrics/Gynecology (E.S., C.J.H., E.A.R.) and Medicine and the General Clinical Research Center (X.C., G.B.), Temple University Hospital, Philadelphia, Pennsylvania.

Address correspondence to Guenther Boden, MD, Temple University Hospital, 3401 North Broad St., Philadelphia, PA 19140.

Received for publication 13 February 1997 and accepted in revised form 7 May 1997.

Abbreviations: CV, coefficient of variation; EGP, endogenous glucose production; FFM, fat-free mass; GIR, glucose infusion rate; G_{Ra}, rate of glucose appearance; G_{Rd}, rate of glucose disappearance; HPL, human placental lactogen; NOGU, nonoxidative glucose utilization; npRQ, nonprotein respiratory quotient; RIA, radioimmunoassay.

RESEARCH DESIGN AND METHODS

Subjects

Six healthy obese (BMI >27.3 kg/m^2 or body fat >30% in the nonpregnant state) pregnant women were studied. Subject age, weight, height, and body composition are shown in Table 1. None of the subjects had a family history of diabetes or any other endocrine disorders, and none were taking any medications. All subjects were seen by a dietitian before the studies to standardize their food intake. Their diet contained a minimum of 250 g of carbohydrate for at least 2 days before the studies. All subjects underwent a standard diabetes screening test (50 g of glucose p.o. followed by measurement of plasma glucose after 1 h) between 24 and 28 weeks gestation. All six subjects had either a normal 1-h glucose challenge test or a 3-h oral glucose tolerance test with all values within the normal range based on the criteria of Carpenter and Coustan (14). None of the women were taking any medications, including any form of hormonal contraception in the postpartum period. The studies were approved by the Temple University Hospital Institutional Review Board, and informed consent was obtained from each subject before the study.

Experimental design

All subjects were studied three times at the General Clinical Research Center at Temple University Hospital; the first time during the second trimester of pregnancy (22.5 ± 2.0 weeks gestation), the second time during the third trimester (36.8 ± 0.9 weeks gestation), and the third time in the postpartum period (15.6 ± 1.4 weeks after delivery) when none of the women were lactating. During the studies, the subjects were reclining in bed. A short polyethylene

Table 1—*Study subjects*

	2nd trimester 22.5 ± 2.0 weeks	3rd trimester 36.8 ± 0.9 weeks	Postpartum 15.6 ± 1.4 weeks	*P* value
Age (years)	27 ± 4.0	—	—	—
Height (cm)	159 ± 1.7	—	—	—
Weight (kg)	76.6 ± 6.2	83.3 ± 7.2	75.5 ± 8.3	NS
BMI (kg/m^2)	30.4 ± 2.8	32.8 ± 3.0	29.9 ± 3.3	NS
Percent fat	37.4 ± 2.5	37.8 ± 3.5	36.7 ± 2.6	NS
FFM	47.3 ± 2.4	50.7 ± 2.0	46.9 ± 2.8	NS

Data are means ± SD. NS, not significant.

catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was kept at ~70°C with a heating blanket to arterialize venous blood (15). After an overnight fast (at ~8:00 A.M.), a 4-h euglycemic-hyperinsulinemic clamp was performed in combination with infusion of stable isotopes (for measurement of glucose turnover) and indirect calorimetry (for estimation of rates of carbohydrate oxidation).

Methods and procedures

Euglycemic-hyperinsulinemic clamp. Regular human insulin (Humulin R, Lilly, Indianapolis, IN) was infused intravenously at a rate of 7 pmol · kg^{-1} · min^{-1} for 4 h starting at 0 min. Glucose concentrations were maintained at ~4.7 mmol/l by a variable rate infusion with 20% glucose, as described (16).

Glucose turnover. Glucose turnover was determined using the stable isotope [6,6-$^2H_2$]glucose as described (17). Briefly, the tracer was infused intravenously for 5.5 h (−90 to 240 min) starting with a bolus of 30 μmol/kg, followed by a continuous infusion of 0.3 μmol · kg^{-1} · min^{-1}. To assure isotope equilibration, the tracer infusion was started 90 min before initiation of the clamp. Blood was drawn at 30-min intervals for determination of [6,6-$^2H_2$]glucose enrichment, which was determined with a gas chromatograph–mass spectrometer (model 4610-B, Finnigan-MAT, San Jose, CA). The penta-acetyl derivative of glucose was measured by the electron impact mode at 70 eV. Ions were measured at m/e 242 and 244, respectively. To avoid changes in isotope enrichment of plasma glucose during the hyperinsulinemia, [6,6-$^2H_2$]glucose was added to the unlabeled glucose, which was infused at variable rates to maintain euglycemia (18). Rates of total body glucose appearance (G_{Ra}) and disappearance (G_{Rd}) were calculated using Steele's equation (19).

Indirect calorimetry. Respiratory gas exchange rates were determined, as previously described (20), before and at 30-min intervals during the clamp with a metabolic measurement cart (Beckman Instruments, Palo Alto, CA). Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in urea nitrogen pool size (21). Rates of protein oxidation were used to determine the nonprotein respiratory quotient (npRQ). Rates of carbohydrate oxidation were determined with the tables of Lusk, which are based on an npRQ of 0.707 for 100% fat oxidation and 1 for 100% carbohydrate oxidation.

Body composition analysis. Skinfold thickness was measured over the triceps, the thigh, and the suprailiac area (22). Duplicate readings were performed at each site to improve the accuracy and reproducibility of the measurements. In addition, body composition was determined with ^2H_2O on the day after the hyperinsulinemic-euglycemic clamp in four of the six subjects (23). The differences in percent body fat between the two methods did not exceed 3%. Anthropometric measurements were used for calculations of FFM and fat mass to maintain consistency.

Fetal assessment. During the second- and third-trimester clamp studies, fetal well-being was assessed every 30 min throughout the insulin infusions by fetal heart rate monitoring. Assessment of amniotic fluid volume, fetal body tone, movement, and breathing (biophysical profile) was done by ultrasound on admission and before discharge. All the women participating in this study delivered a healthy baby.

Endogenous glucose production (EGP). Most (>75%) of the EGP comes from the liver, while the kidneys at times may produce a small amount of glucose (24). EGP was calculated as the difference between the isotopically determined rates of glucose appearance (G_{Ra}) and the glucose infusion rates (GIR) needed to maintain euglycemia during hyperinsulinemia (EGP = G_{Ra} − GIR).

Nonoxidative glucose utilization (NOGU). NOGU, which consists of glycogen synthesis plus lactate/alanine production, was calculated as the difference between the isotopically determined rates of glucose disappearance (G_{Rd}) and carbohydrate oxidation (NOGU = G_{Rd} − carbohydrate oxidation).

Analytical procedure

Plasma glucose was measured with a glucose analyzer (Beckman). Plasma free insulin was determined by radioimmunoassay (RIA) after polyethylene glycol precipitation using an antiserum with minimal (<0.2%) cross-reactivity with proinsulin (Linco Research, St. Charles, MO). Plasma human placental lactogen (HPL) and cortisol were determined by RIA with kits from DPC-Diagnostic Products, Los Angeles, CA.

Statistical analysis

All data were expressed as the mean ± SE. Rates of glucose uptake, carbohydrate oxidation, and endogenous glucose production were expressed per kilogram fat-free mass (FFM) since most of the intravenously infused glucose is metabolized in muscle (25). Statistical significance was assessed using analysis of variance (ANOVA) with repeated measures and Student's two-tailed paired or unpaired *t* test, where applicable.

RESULTS

Plasma insulin and glucose (Fig. 1)

Insulin infusion raised plasma insulin concentrations from 82 ± 9 to 457 ± 52 pmol/l in the second trimester; from 104 ± 26 to 531 ± 86 pmol/l in the third trimester; and from 103 ± 22 to 604 ± 121 pmol/l in the postpartum period. The differences in both preclamp and clamp insulin concentrations were not statistically significant for the three study periods.

Preclamp glucose concentrations were 4.3 ± 0.05 mmol/l (second trimester), 4.5 ± 0.2 mmol/l (third trimester), and 4.9 ± 0.2 mmol/l (postpartum). These differences

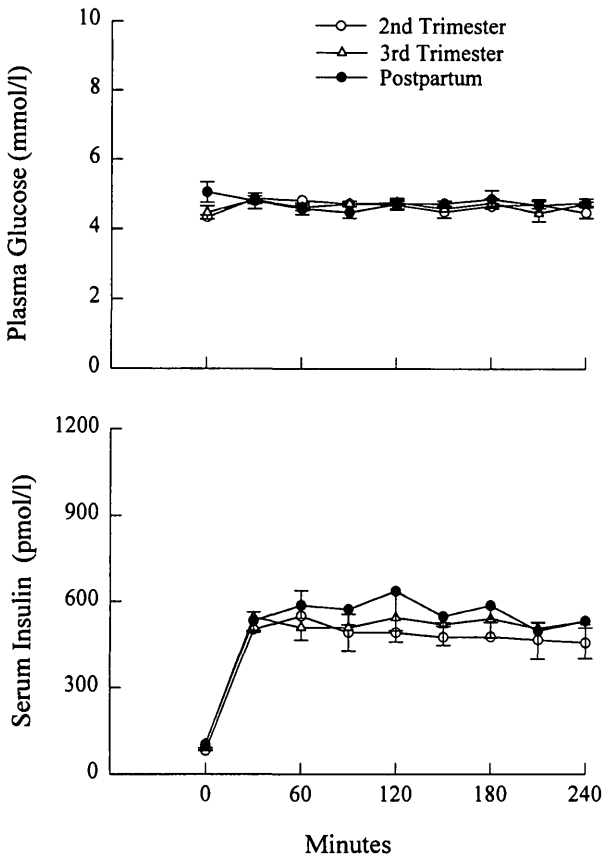

Figure 1—*Plasma glucose and insulin concentrations before and during euglycemic-hyperinsulinemic clamping in six obese healthy women during the second (22.5 ± 2.0 weeks; ○) and third (36.8 ± 9 weeks; △) trimesters of pregnancy and postpartum (15.6 ± 1.4 weeks after delivery; ●).*

were not statistically significant. Plasma glucose was clamped at ~4.7 ± 0.1 mmol/l (coefficient of variation [CV] 7.4%) in all three studies.

Glucose uptake (G_{Rd}) (Fig. 2). In response to 240 min of hyperinsulinemia, G_{Rd} increased from 12.3 ± 0.5 to 55.1 ± 6.7 μmol · kg^{-1} FFM · min^{-1} (459%; $P < 0.01$) during the second trimester; from 13.7 ± 1.2 to 40.9 ± 5.6 μmol · kg^{-1} FFM · min^{-1} during the third trimester (283%; $P < 0.01$); and from 16.6 ± 1.6 to 65.3 ± 9.0 μmol · kg^{-1} FFM · min^{-1} (415%; $P < 0.01$) during the postpartum period. The difference in insulin-stimulated G_{Rd} between the second trimester and postpartum (55.1 vs. 65.3 μmol · kg^{-1} FFM · min^{-1}) was not significant. However, the insulin-stimulated increase in G_{Rd} was significantly reduced during the third trimester when compared with the postpartum state (−40%, $P < 0.05$) and with the second trimester of pregnancy (−28%, $P < 0.05$).

Carbohydrate oxidation. In response to hyperinsulinemia, rates of carbohydrate oxidation increased from 5.9 ± 1.6 to 15.2 ± 1.4 μmol · kg^{-1} FFM · min^{-1} (258%, $P < 0.01$) during the second trimester of pregnancy; from 6.3 ± 1.4 to 8.2 ± 1.9 μmol · kg^{-1} FFM · min^{-1} (146%, NS) during the third trimester; and from 5.6 ± 1.1 to 17.8 ± 2.8 μmol · kg^{-1} FFM · min^{-1} (318%, $P < 0.01$) during the postpartum studies. The difference in insulin-stimulated carbohydrate oxidation between the second trimester and postpartum studies (15.2 vs. 17.8 μmol · kg^{-1} FFM · min^{-1}) was not statistically significant. During the third trimester, however, the insulin-stimulated increase in carbohydrate oxidation was significantly reduced compared with the postpartum state (8.2 vs. 17.8 μmol · kg^{-1} FFM · min^{-1}; $P < 0.05$) and to the second trimester (8.2 vs. 15.2 μmol · kg^{-1} FFM · min^{-1}, $P < 0.05$) (Fig. 2, bottom).

Endogenous glucose production (EGP) (Fig. 3). Postpartum, insulin suppression of EGP was nearly complete after 2 h (from 15.7 ± 1.4 to 3.7 ± 1.0 μmol · kg^{-1} FFM · min^{-1} (−77%). Similarly, during the second trimester, EGP decreased from 12.0 ± 0.6 to 2.5 ± 0.9 μmol · kg^{-1} FFM · min^{-1} after 2 h (−79%). In contrast, during the third trimester, the inhibition of EGP was significantly delayed (overall comparison of curves, third vs. second trimester, $P < 0.05$; third trimester vs. postpartum, $P < 0.03$). After 2 h, EGP had decreased by only 39% (from 13.9 ± 1.4 to 8.6 ± 1.0 μmol · kg^{-1} FFM · min^{-1}, $P < 0.01$). However, after 4 h, EGP was suppressed similarly during the second and third trimester and postpartum (to 1.9 ± 0.9, 3.2 ± 1.8 and 2.1 ± 0.7 μmol · kg^{-1} FFM · min^{-1}, respectively).

Nonoxidative glucose uptake (NOGU). NOGU increased from 5.9 ± 1.6 to 40.0 ± 5.9 μmol · kg^{-1} FFM · min^{-1} (678%) during the second trimester, from 8.4 ± 1.4 to 30.6 ± 4.4 μmol · kg^{-1} FFM · min^{-1} (364%) during the third trimester, and from 10.7 ± 0.4 to 51.7 ± 5.7 μmol · kg^{-1} FFM · min^{-1} (483%) postpartum.

Plasma HPL and cortisol. Fasting plasma HPL levels were 5.4 ± 0.8 nmol/l postpartum, 87 ± 19 nmol/l during the second trimester, and 286 ± 25 nmol/l during the third trimester. Fasting plasma cortisol levels were 105 ± 20 nmol/l postpartum, 401 ± 50 nmol/l during the second trimester, and 627 ± 60 nmol/l during the third trimester. Thus, plasma HPL levels were 53-fold higher during the third trimester compared with the nonpregnant state, while plasma cortisol levels were 6-fold higher.

CONCLUSIONS

Peripheral insulin sensitivity

It was the objective of this study to longitudinally examine changes in insulin sensitivity during and after pregnancy in obese healthy women. Six women were studied during the second and third trimesters of their pregnancies and again 3–4 months after delivery when they had stopped lactating and had resumed their menstrual cycles. All had normal glucose tolerance tests when tested during the third trimester, and all delivered healthy babies. The results obtained showed that in these obese women, the ability of physiological hyperinsulinemia (500–600 pmol/l) to stimulate peripheral glucose uptake (i.e., mainly uptake into muscle) (25) had changed little (−15%, NS) during the second trimester but was reduced by ~40% ($P < 0.05$) during the third trimester when compared with the nonpregnant (postpartum) state.

These results in obese women can be compared with data from the only other

longitudinal study, published by Catalano et al. (6). These researchers have examined changes in insulin sensitivity in six healthy normal-weight women who underwent 2-h euglycemic-hyperinsulinemic clamping before and during early (12–14 weeks) and late (32–34 weeks) pregnancy (6,26). After an overnight fast, the normal-weight, nonpregnant women (BMI 20.3) of Catalano et al. were ~40% more insulin sensitive than this study's obese (BMI 30.4) nonpregnant women (insulin-stimulated glucose uptake 58.3 vs. 41.6 μmol · kg^{-1} FFM · min^{-1} at 120 min), most likely because of the difference in body weight because obesity is known to be associated with increased insulin resistance (27).

In response to hyperinsulinemia, there was a severe reduction in insulin-stimulated glucose uptake (i.e., an increase in peripheral insulin resistance) during late pregnancy in both obese (this study) and normal-weight pregnant women (26). Interestingly, however, the third-trimester loss in insulin sensitivity (compared with the nonpregnant state) appeared to have been greater in normal-weight women, where insulin-stimulated glucose uptake decreased from 58.3 to 25.5 μmol · kg^{-1} FFM · min^{-1} (−56%) (26) than in our obese women, where it decreased from 41.7 to 31.1 μmol^{-1} · kg FFM · min^{-1}, (−25%). It is possible, however, that the difference was due to Catalano et al. studying women before they became pregnant, while we studied women postpartum.

The use of indirect calorimetry in this study allowed evaluation of intracellular glucose utilization. The data showed that intracellular glucose utilization was essentially the same during the second trimester and postpartum, with ~27% of glucose uptake being terminally oxidized and the balance going into nonoxidative glucose utilization (consisting of glycogen synthesis, lactate, and alanine production). We have previously reported comparable intracellular glucose distribution rates in healthy nonpregnant subjects (28). The normal intracellular glucose utilization pattern observed during the second trimester, however, changed during the third trimester, when insulin was no longer able to significantly increase carbohydrate oxidation (from 6.3 to 8.2 μmol · kg^{-1} FFM · min^{-1}; NS). This resulted in a decrease of the percentage of glucose uptake being oxidized, which was out of proportion to the decrease in glucose uptake (−80% vs. −40%, $P < 0.02$).

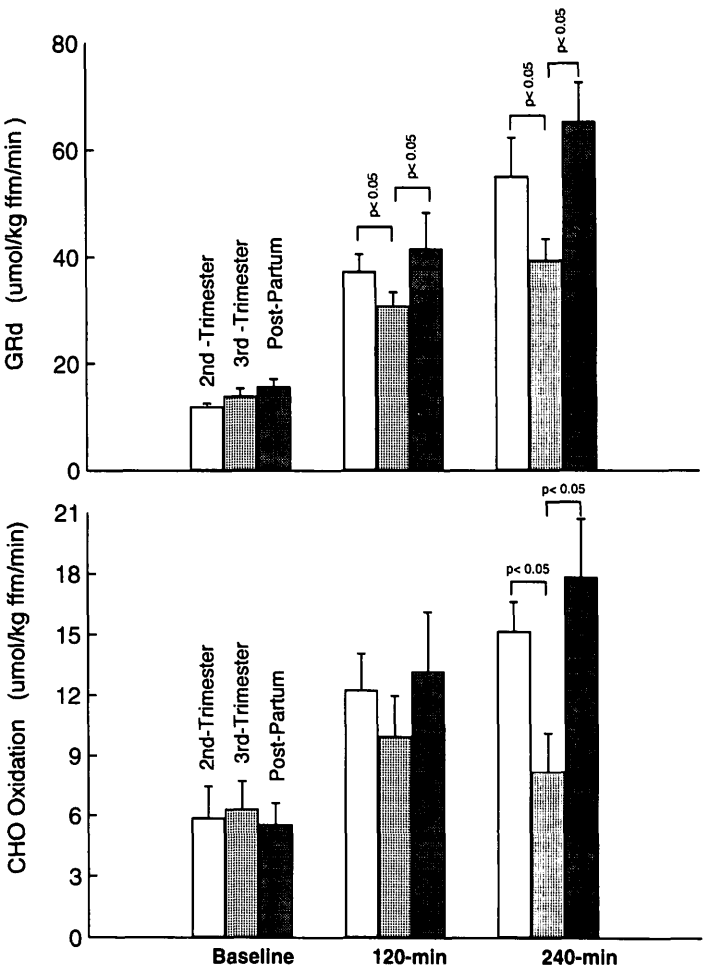

Figure 2—*Rates of whole-body glucose disappearance (G_{Rd}) and carbohydrate oxidation in six obese healthy women before (baseline) and after 120 min and 240 min of euglycemic-hyperinsulinemic clamping during the second and third trimesters of pregnancy and postpartum. Shown are mean ± SE. Data are expressed as micromoles per kilogram of fat-free mass per min.*

Thus, there appeared to be two distinct alterations affecting peripheral insulin sensitivity in obese pregnant women. The first alteration, barely noticeable during the second trimester, became much more severe during the third trimester, reducing peripheral insulin sensitivity by ~40%. The proportional decrease in glucose uptake and carbohydrate oxidation in the second trimester suggested a defect proximal to the carbohydrate oxidation step, perhaps at the level of glucose transport and/or phosphorylation. The cause or causes are not entirely clear. The parallel development of insulin resistance and increases in blood levels of HPL and cortisol, hormones with strong anti-insulin action (29–31), suggested that HPL, cortisol, and perhaps other diabetogenic hormones, including progesterone and estrogen may have been responsible for much of the observed insulin resistance. In support of this, administration of these gestational hormones has been shown to increase insulin release and to produce elevated insulin/glucose ratios (32–34).

The second alteration, a decrease in carbohydrate oxidation exceeding the decrease in glucose uptake, became detectable only during the third trimester. Its cause is also unknown. It did not seem to be caused by an increase in plasma FFA, since FFA concentrations were comparable during the second and third trimester (basal, 624 ± 39 vs. 608 ± 48 μmol/l; clamp, 263 ± 32 vs. 299 ± 27 μmol/l).

Hepatic insulin sensitivity

In addition, our data revealed an alteration in hepatic insulin action. There were only

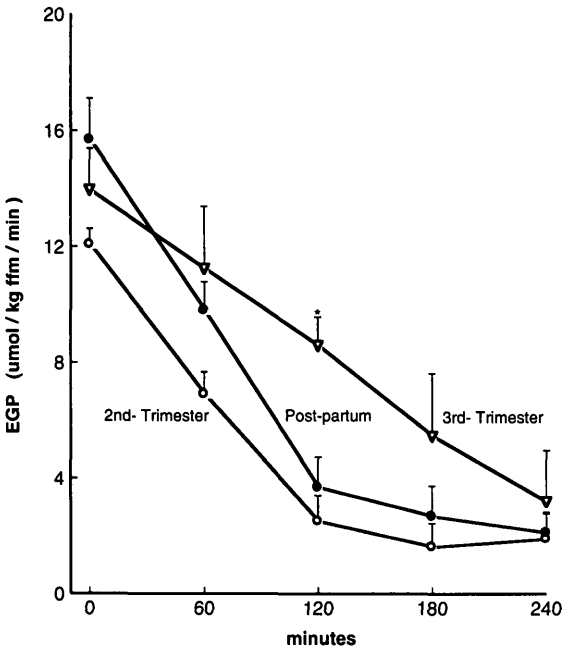

Figure 3—*Rates of endogenous glucose production before (0 min) and during euglycemic-hyperinsulinemic clamping in six obese healthy women during the second and third trimesters of pregnancy and postpartum.*

small and nonsignificant changes in basal EGP comparing the second and third trimester with the postpartum state. After 2 h of hyperinsulinemia, however, EGP was significantly less inhibited during the third trimester (from 13.9 to 8.6 μmol · kg^{-1} FFM · min^{-1}, −39%) when compared with the second trimester (from 12.0 to 2.5 μmol · kg^{-1} FFM · min^{-1}, −79%) or with the nonpregnant (postpartum) state (from 15.7 to 3.7 μmol · kg^{-1} FFM · min^{-1}, −77%). After 4 h of hyperinsulinemia, however, this defect had disappeared, i.e., hepatic insulin resistance (>75% of EGP comes from the liver) had been overcome by longer exposure to hyperinsulinemia.

In contrast, Catalano et al. found that in normal-weight pregnant women, hyperinsulinemia (500–600 pmol/l for 120 min) suppressed EGP completely (by 89, 93, and 95% prepregnancy, during early and late gestation, respectively [26]).

Physiological considerations

The development of peripheral and hepatic insulin resistance after midpregnancy can be seen as an effort by the obese women to adapt to the fuel needs of the rapidly growing fetus (35). During the third trimester of pregnancy, glucose uptake by the fetus has been estimated to be ~33 μmol · kg^{-1} · min^{-1} (36). To satisfy this additional need, the obese women sharply increased peripheral insulin resistance, thus reducing maternal glucose utilization. They also increased hepatic insulin resistance albeit to a lesser extent, which probably resulted in increased production of glucose. Moreover, by decreasing terminal carbohydrate oxidation, they shunted much of the glucose entering muscle into either glycogen or into lactate and thus made it available to be recycled into glucose via the Cori-cycle.

Acknowledgments— Supported by National Institutes of Health Grants R01-AG-07988 (G.B.), R01-AA-10221 (G.B.), RR-349 (General Clinical Research Center), a mentor-based postdoctoral fellowship grant from the American Diabetes Association (G.B.) and a grant from the March of Dimes 6-FY94-0300 (E.A.R.).

We thank the nurses of the General Clinical Research Center for assistance with the clinical studies, Karen Davis and Maria Mozzoli for technical assistance, and Constance Harris for typing the manuscript.

References

1. Bleicher SJ, O'Sullivan JB, Freinkel N: Carbohydrate metabolism in pregnancy. *N Engl J Med* 271:866–872, 1964
2. Lind T, Billewicz W, Brown G: A serial study of changes occurring in the oral glucose tolerance test during pregnancy. *J Obstet Gynaecol Br Commonw* 80:1033–1039, 1973
3. Cousins L, Rigg L, Hollingsworth D, Brink G, Aurand J, Yen SS: The 24-hour excursions and diurnal rhythm of glucose insulin and C-peptide in normal pregnancy. *Am J Obstet Gynecol* 136:483–488, 1980
4. Spellacy W, Goetz F: Plasma insulin in normal late pregnancy. *N Engl J Med* 268:988–991, 1963
5. Ryan EA, Sullivan MJ, Skyler JS: Insulin action during pregnancy: studies with the euglycemic clamp technique. *Diabetes* 34:380–389, 1985
6. Catalano PM, Tyzibir ED, Roman NM, Amini SB, Sims EAH: Longitudinal changes in insulin resistance in non-obese pregnant women. *Am J Obstet Gynecol* 165:1667–1772, 1991
7. Schmitz O, Klebe J, Moller S, Arnfred J, Hermansen K, Orskov K, Beck-Nielsen H: In vivo insulin action in type 1 (insulin-dependent) diabetic pregnant women as assessed by the insulin clamp technique. *J Clin Endocrinol Metab* 61:877–881, 1985
8. Buchanan TA, Metzger BE, Freinkel N, Bergman RN: Insulin sensitivity and beta-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. *Am J Obstet Gynecol* 162:1008–1014, 1990
9. Campbell PJ, Gerich JE: Impact of obesity on insulin action in volunteers with normal glucose tolerance: demonstration of a threshold for the adverse effect of obesity. *J Clin Endocrinol Metab* 70:1114–1118, 1990
10. Calandra C, Abell DA, Beischer NA: Maternal obesity in pregnancy. *Obstet Gynecol* 57:8–12, 1981
11. Johnson JW, Longmate JA, Frentzen B: Excessive maternal weight and pregnancy outcome. *Am J Obstet Gynecol* 167:353–370, 1992
12. Bray GA: Obesity increases risk for diabetes. *Int J Obes* 16 (Suppl. 4):S13–S17, 1992
13. Modan M, Karasik A, Halkin H, Fuchs Z, Lusky A, Shitrit A, Modan B: Effect of past and concurrent body mass index on prevalence of glucose intolerance and type 2 (non-insulin-dependent) diabetes and on insulin response: the Israel Study of Glucose Intolerance, Obesity and Hypertension. *Diabetologia* 29:82–89, 1986
14. Carpenter MW, Coustan DR: Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 144:768–773, 1982
15. Abumrad NN, Rabin D, Diamond MP, Lacy WW: Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30:936–940, 1981
16. Boden G, DeSantis R, Chen X, Morris M, Badoza F: Glucose metabolism and leg

blood flow after pancreas/kidney transplantation. *J Clin Invest* 76:1229–1233, 1993
17. Boden G, Chen X, DeSantis R, White J, Mozzoli M: Effects of ethanol on carbohydrate metabolism in the elderly. *Diabetes* 42:28–34, 1993
18. Molina JM, Baron AD, Edelman SV, Brechtel G, Wallace P, Olefsky JM: Use of a variable tracer infusion method to determine glucose turnover in humans. *Am J Physiol* 258:E16–E23, 1990
19. Steele R, Wall JS, DeBodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–24, 1956
20. Owen OE, Trapp VE, Reichard GA Jr, Mozzoli MA, Smith R, Boden G: Effects of therapy on the nature and quantity of fuels oxidized during diabetic ketoacidosis. *Diabetes* 29:365–372, 1980
21. Tappy L, Owen OE, Boden G: Effect of hyperinsulinemia on urea pool size and substrate oxidation rates. *Diabetes* 37:1212–1216, 1988
22. Diaz E, Villar J, Immin KM, Gonzalez T: Bioimpedance or anthropometry? *Eur J Clin Nutr* 43:1219–1237, 1989.
23. Mendez J, Prokop E, Picon-Reategui E, Akers R, Buskirk ER: Total body water by D_2O dilution using saliva samples and gas chromatography. *J Appl Physiol* 28:354–357, 1970
24. Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierez O, Gerich J: Uptake and release of glucose by human kidney. *J Clin Invest* 96:2528–2533, 1995
25. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic femoral venous catheterization. *Diabetes* 30:1000–1007, 1981
26. Catalano PM, Tyzbir ED, Wolfe RR, Roman NM, Amini SB, Sims EAH: Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am J Obstet Gynecol* 167:913–919, 1992
27. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, DelPrato S, DeFronzo RA: Obesity and insulin resistance in humans: a dose-response study. *Metabolism* 39:452–459, 1990
28. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty-acid induced inhibition of glucose uptake. *J Clin Invest* 93: 2438–2446, 1994
29. Beck P, Daughaday WH: Human placental lactogen: studies of its acute metabolic effects and disposition in normal man. *J Clin Invest* 46:103–110, 1967
30. Turtle JR, Kipnis DM: The lipolytic action of human placental lactogen in isolated fat cells. *Biochim Biophys Acta* 144:583–593, 1967
31. Kalkhoff RK, Richardson BL, Beck P: Relative effects of pregnancy, human placental lactogen and prednisolone on carbohydrate tolerance in normal and subclinical diabetic subjects. *Diabetes* 18:153–163, 1969
32. Burke CW, Roulet F: Increased exposure of tissues to cortisol in late pregnancy. *Br Med J* 1:657–659, 1970
33. Costrini NV, Kalkhoff RK: Relative effects of pregnancy, estradiol, and progesterone on plasma insulin and pancreatic islet insulin secretion. *J Clin Invest* 50:992–997, 1971
34. Rushakoff RJ, Kalkhoff RK: Effects of pregnancy and sex steroid administration on skeletal muscle metabolism in the rat. *Diabetes* 30:545–550, 1981
35. Boden G: Fuel metabolism in pregnancy and in gestational diabetes mellitus. *Obstet Gynecol Clin North Am* 23:1–10, 1996
36. Crenshaw C Jr: Fetal glucose metabolism. *Clin Obstet Gynecol* 13:579–585, 1970