Birth Weight and Fetal Mortality in Pregnant Subdiabetic Rats

Arnold Lazarow, M.D., Ph.D., Jae Nam Kim, M.D., Ph.D., and Lemen J. Wells, Ph.D., Minneapolis

It has been reported that diabetic women frequently give histories of fetal overweight and of increased fetal mortality in pregnancies occurring many years before the development of manifest diabetes.[1] Wilkerson studied the glucose tolerance in a large series of pregnant women.[2] In those instances where he observed abnormal carbohydrate tolerance during pregnancy, he found that newborns weighing nine pounds or more occurred three times as frequently as in the group with normal carbohydrate tolerance.

Lazarow reported that subdiabetes in rats could be produced by injecting subthreshold doses of alloxan.[3] Although these rats had an abnormal tolerance to glucose, their fasting and postprandial blood sugars were normal. They did not show any glycosuria.

In two thirds of these animals, subdiabetes persisted for a period up to a year and more. One third of these animals developed manifest diabetes (hyperglycemia and glycosuria) during the first year.

The present study was undertaken to determine whether pregnant subdiabetic rats likewise show a high incidence of stillborns and overweight newborns.

METHODS

The animals used were albino rats of a subline of the Sprague-Dawley strain (Holtzman). Most of the rats were virgin females, 120 to 150 days in age, weighing between 230 and 280 gm. The rats were fed a standard diet of Purina fox chow. Food and water were supplied ad libitum. The experimental and control animals were maintained under similar conditions.

Production of subdiabetes: Alloxan was dissolved in distilled water to make a 2 or 4 per cent solution immediately prior to injection. Nonpregnant females were injected intravenously with alloxan in a dose of 25 or 30 mg. per kg. of body weight (Lazarow and Palay).[4] A few rats were injected with alloxan on the twelfth day of pregnancy. Those rats which did not show either hyperglycemia or glycosuria were subjected to glucose tolerance tests. Animals were fasted sixteen to eighteen hours and injected intravenously with a 30 per cent glucose solution in a dose of 3 gm. per kg. of body weight. Blood sugars (BS) were determined by the Folin-Malmros micromethod.[5] The diabetic index (I_D) which is a measure of the degree of abnormality of glucose tolerance was determined for each test, using the following formula (Coupland, Davidson and Lazarow).[6]

$$I_D = \frac{\text{1 hr. experimental BS}}{\text{1 hr. average normal BS}} \times \frac{\text{2 hr. experimental BS}}{\text{2 hr. average normal BS}}$$

In forty-two tolerance tests carried out in twelve normal rats, the average one- and two-hour blood sugar values were 175.2 and 111.9 mg. per cent, respectively, and those values were used in calculating the diabetic index. The index determined in normal rats equals 1.00 plus or minus the standard deviation of 0.30 (Coupland, Davidson, and Lazarow).[7]

All fourteen subdiabetic rats had diabetic indices greater than 2.0, i.e., more than three standard deviations greater than normal. In eleven of the rats used, subdiabetes was induced prior to pregnancy; in three, subdiabetes was induced on the twelfth day of pregnancy. Since the average birth weights and fetal mortalities were similar in the two subdiabetic groups, all of the subdiabetic rats were treated as a single group.

Blood sugar was determined at frequent intervals in all animals. The urine sugar was determined by the caramelization method of Somogyi.[8] The twenty-four-hour urinary excretion and the urine sugar level were determined at various times during the course of pregnancy, in order to be certain that none of the sub-

From the Department of Anatomy, University of Minnesota, Minneapolis 14, Minnesota. Dr. Jae Nam Kim was a Fellow of the International Cooperation Administration, 1955-1959. His present address is: Department of Anatomy, Seoul National University Medical School, Seoul, Korea.

diabetic rats developed manifest diabetes during the course of study.

Breeding: Vaginal smears were made daily in the experimental groups. An animal showing estrus was placed with males of the same strain and watched until copulation took place. Time of mating was recorded. After matings, females were caged separately.

Length of gestation: Beginning at twenty-one days and fifteen hours postcoitum, i.e., one hour prior to the earliest time of delivery in control pregnancies in this strain of rats (Wells),[9] the observations were made at intervals of fifteen to sixty minutes until spontaneous delivery. Thus, accurate observations on litter size and on stillborns could be made. Also, the length of gestation (time between witnessed mating and witnessed delivery of the first young) was accurate to less than one hour.

Weighing of newborns: Newborn rats were weighed individually immediately after birth and before the suckling of milk. In weighing a newborn, the chainomatic balance was read to the closest milligram.

RESULTS

The number of animals involved in each range of diabetic indices is given in table 1.

TABLE 1

Diabetic indices (I_D) of fourteen subdiabetic rats

Range of I_D	Number of animals*
2.00 to 2.50	7
2.51 to 3.00	2
3.01 to 3.50	2
3.51 to +	4

*One of the fourteen pregnant females produced two litters, hence is listed twice.

Birth weight: In analyzing the data, the average birth weight of a litter was used to calculate the standard deviations and the P values. Figure 1 shows that in the normal (control) group the mean birth weight of thirty-nine litters was 6.224 gm.; the standard error of the mean was 0.0815 gm. By contrast, in the subdiabetic group the mean birth weight of fifteen litters was 6.691 gm.; the standard error of the mean was 0.0918 gm. Thus, the mean birth weight of newborns born to subdiabetic mothers was 7.5 per cent greater than that of normal controls. This increase was statistically significant (P less than 0.001). There were no significant differences between the normal and subdiabetic groups in the length of gestation and in litter size.

When the birth weight of individual newborns was

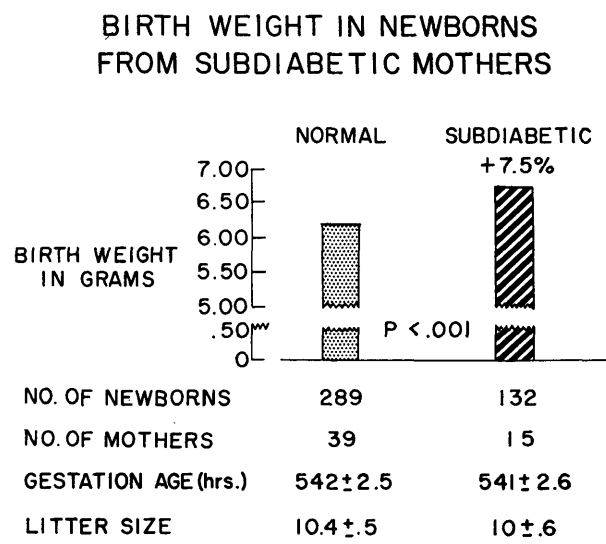

FIGURE 1

taken as an independent variable, the mean birth weight in 289 newborns from thirty-nine mothers was 6.274 gm. One standard deviation of the mean was 0.555 gm. Figure 2 shows the percentages of newborns whose weights exceeded the mean birth weight of the control group by 1, 2 and 3 standard deviations, respectively. In the normal control group, 14.2 per cent (forty-one out of 289 newborns) weighed 6.829 gm. or more (greater than one standard deviation from the mean); in the subdiabetic group, 30.3 per cent (forty out of 132 newborns) fell in the same range. The corresponding percentages for the normal and subdiabetic groups were 1 per cent and 3.8 per cent respectively at two standard deviations and 0 per cent and 1.5 per cent at three standard deviations. It should be noted that subdiabetic mothers produced overweight newborns 3.8 times more frequently than normal mothers (greater than two or more standard deviations). Furthermore, it is interesting to note that two out of 132 newborns from subdiabetic mothers weighed 7.939 gm. or more (more than three standard deviations greater than normal); whereas none of 289 newborns from normal mothers weighed that much.

Fetal mortality: In figure 3, the frequencies of stillbirths and neonatal deaths (newborns which had reflexes but which failed to breathe or survive) are recorded. The mortality in the normal group was 5.9 per cent (twenty-nine out of 289 newborns). By contrast, the mortality in the subdiabetic group was 18.7 per cent (twenty-eight out of 150 newborns). This increase in fetal mortality was statistically significant (P less than 0.001).

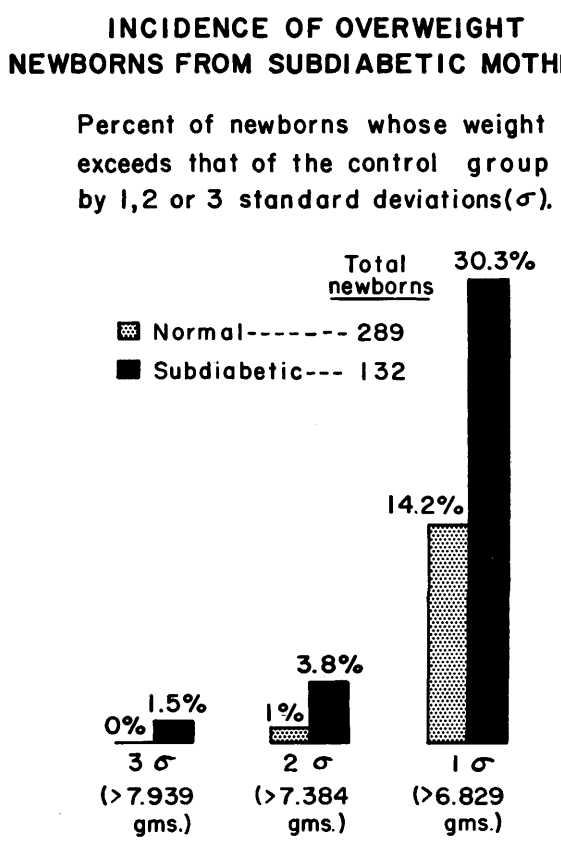

FIG. 2. The three weight limits at the bottom of the figure were calculated from the data on normal newborns (σ = standard deviation from the mean).

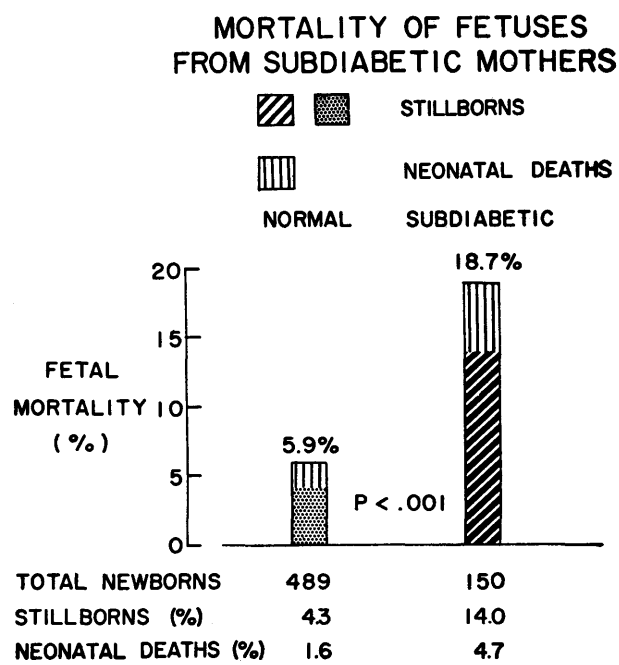

FIG. 3. Neonatal deaths are the deaths of newborns that had reflexes but failed to breathe and/or live one hour.

DISCUSSION

Overweight newborns (7.384 gm. or more) occurred about four times more frequently in newborns from subdiabetic mothers than in those from normal mothers. It should be noted that the increased birth weight in newborns from subdiabetic mothers occurred without any concomitant increase in the length of the gestation period. It has been reported that in the rat there is a positive correlation between the gestation age and the birth weight (Kim).[10]

In physical characteristics, although larger than those from control mothers, newborns from subdiabetic rats did not show evident signs of edema or of excessive fat accumulation. These findings suggest that subdiabetes stimulates the growth of fetuses in utero.

Miller, Hurwitz and Kuder,[1] defining an overweight human baby as one who weighs more than 5 kg., reported that the incidence of overweight babies born to prediabetic women is 3.9 per cent, whereas that in newborns from nondiabetic mothers is 0.07 per cent. Likewise, the average weight in infants born of prediabetic women was significantly greater than that in infants born of nondiabetic mothers. The increased birth weight and the increased fetal mortality were reported as early as one to three decades prior to the clinical onset of diabetes.[1,11,12]

Subdiabetic rats were apparently healthy, and did not excrete an excessive amount of urine. It should be noted that a significant increase in the incidence of stillbirths was observed in these subdiabetic rats at spontaneous delivery, even though their blood sugars were normal and they did not show glycosuria. It is of interest to point out that the mortality in the newborns from subdiabetic rats is similar to that reported for newborn infants from prediabetic women, i.e., 18.7 per cent and 14 per cent, respectively.[1] Similarly, the mortality rates for the normal rat and the normal human are 5.9 and 5 per cent, respectively (figure 3).[12]

Mechanisms which increase the fetal weight and fetal mortality are unknown. It is of interest to point out that the experimental induction of subdiabetes in the rat produces an increased fetal weight and increased fetal mortality. In the human, both maternal and paternal genetic factors are thought to contribute to fetal overweight.[12,13] If the mechanism by which subdiabetes in the rat produces increased fetal weight and fetal mortality can be determined, it may be possible to prevent these complications in the prediabetic human.

SUMMARY AND CONCLUSIONS

The effects of subdiabetes in the mother on fetal weight and fetal mortality were studied. Pregnancy in the subdiabetic rats was associated with a 7.5 per cent increased birth weight which was statistically significant —P less than 0.001; this occurs without any concomitant increase in the gestation period. The frequency of overweight newborns in the subdiabetic rats was severalfold greater than that in the normal rats. The fetal mortality in the subdiabetic group was three times greater than that in the normal controls—P less than 0.001.

SUMMARIO IN INTERLINGUA

Peso Natal e Mortalitate Fetal in Subdiabetic Rattos Pregnante

Esseva studiate le effecto de subdiabete in le ratta matre super le peso natal e le mortalitate fetal de su prole. Pregnantia in rattas subdiabetic esseva associate con un augmento de 7,5 pro cento in le peso natal. Isto es statisticamente significative—con un valor pro p de minus que 0,001. Il non occurre un concomitante extension del periodo gestatori. Le frequentia de neonatos a peso excessive ab rattas subdiabetic esseva plure vices plus grande que in rattas normal. Le mortalitate fetal in le gruppo de matres subdiabetic esseva tres vices plus alte que in le normal animales de controlo. Isto corresponde a un valor pro p de minus que 0,001.

ACKNOWLEDGMENT

This study was aided by grants from the Medical Research Fund of the Graduate School and Grants A-1659, A-1244 and A-1887 from National Institute of Arthritis and Metabolic Diseases, Public Health Service.

REFERENCES

[1] Miller, H. C., Hurwitz, D., and Kuder, K.: Fetal and neonatal mortality in pregnancies complicated by diabetes mellitus. J.A.M.A. *124*:271-75, 1944.

[2] Wilkerson, H. L. C., and Remein, Q. R.: Studies of abnormal carbohydrate metabolism in pregnancy. The significance of impaired glucose tolerance. Diabetes *6*:324-29, 1957.

[3] Lazarow, A.: Spontaneous recovery from alloxan diabetes in the rat. Diabetes *1*:363-72, 1952.

[4] Lazarow, A., and Palay, S. L.: Production and course of alloxan diabetes in the rat. J. Lab. & Clin. Med. *31*:1004-15, 1946.

[5] Folin, O., and Malmros, H.: An improved form of Folin's micro method for blood sugar determination. J. Biol. Chem. *83*:115-20, 1929.

[6] Coupland, R. E., Davidson, J., and Lazarow, A.: A diabetic index in the rat as determined by glucose tolerance tests. Anat. Rec. *124*:394, 1956.

[7] Coupland, R. E., Davidson, J., and Lazarow, A.: Observations to be published.

[8] Somogyi, M.: A rapid method for the estimation of urine sugar. J. Lab. & Clin. Med. *26*:1220-23, 1940-1941.

[9] Wells, L. J.: Subjection of fetal rats to surgery and repeated subcutaneous injections: method and survival. Anat. Rec. *108*:309-32, 1950.

[10] Kim, J. N.: The effects of experimental diabetes and subdiabetes on the offspring of rats. Ph.D. Thesis, p. 41, Univ. of Minnesota Library, 1959.

[11] Miller, H. C.: The effect of prediabetic state on the survival of the fetus and the birth weight of the newborn infants. New England J. Med. *233*:376-78, 1945.

[12] Jackson, W. P. U.: Studies in prediabetes. Brit. M. J. *2*:690-96, 1952.

[13] Jackson, W. P. U.: The prediabetic syndrome; large babies and the (pre)diabetic father. J. Clin. Endocrinol. *14*:177-83, 1954.

The arteriovenous differences in fasting subjects (*Nutrition Reviews 16*:102, 1958) indicated that the source of the circulating unesterified fatty acid was adipose tissue. Gordon and Cherkes calculated that if the adipose tissue were the primary source of the unesterified fatty acids, and if that tissue were to account for a significant fraction of the total metabolism of the rat, the adipose tissue should liberate approximately 100 micromoles of unesterified fatty acids per gram of tissue per hour. This rate is almost twenty times greater than that seen in any of their fasting rats (6.4 μM). They suggest that the low rate of unesterified fatty acid production in the in vitro experiments stems from the poor circulation, and that when the blood supply is intact the rate might be more nearly sufficient to account for the energy requirements. Although the amount of insulin incubated with the adipose tissue was at a concentration two orders of magnitude higher than that effective in vivo, they still felt their results suggested that insulin acted directly on the adipose tissue rather than some other tissue or organ.

Additional evidence suggesting that certain tissues liberate, while others utilize, unesterified fatty acids has been presented by P. S. Roheim and J. J. Spitzer (*Am. J. Physiol. 195*:288, 1958). They used sixteen normal dogs that had been fasted over night. In practically all cases there was a higher concentration of unesterified fatty acids in femoral vein blood than in femoral arterial blood. These differences ranged up to 0.49 μM. per liter, with most of them close to 0.15. The investigators interpreted this finding as indicating the release of lipids by the adipose tissue in the legs. There were no differences in the arterial and venous blood insofar as triglycerides, total and free cholesterol, or phospholipids were concerned.

From *Nutrition Reviews,* Vol. 17, No. 8, p. 242, August 1959.