CHANGES OF THE LEVELS OF BLOOD GLUCOSE DURING PREGNANCY IN THE RAT

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The increased utilization of lipids during pregnancy, expressed as an increase of lipids concentration in the blood, liver and kidney as well as a decrease of depot fat, has been demonstrated by Boyd (1), McKay (2) and Scow *et al.* (3). The hypoglycemic response of the fasted pregnant rats associated with the appearance of ketosis has also been shown by Scow *et al.* (3). Apart from these metabolic changes during pregnancy, Garner *et al.* (4) have reported that the level of blood glucose is lower in the rat fed a high-fat diet than in the rat fed a high-carbohydrate diet. These findings allow the assumption that the utilization of glycogen is preceded that of lipids during the fasting by the mechanism of depressed glucose utilization, and permit elucidation of the interaction between glucose and lipid metabolism.

In the present experiments, the influences of pregnancy on the carbohydrate metabolism were observed as changes of the level of blood glucose and tissue glycogen during pregnancy as well as after delivery. The mode of glycogen utilization at the various term of pregnancy and postparturition was investigated by means of the glucose tolerance test. Furthermore, the effects of lipid feeding on the level of blood glucose during pregnancy and after delivery were studied in the rats fed a high-fat diet.

METHODS

Approximately 80 female Wistar rats, weighing 160 to 210 g at the beginning of the experiments, were used. They were housed individually at the room temperature of $22\pm 2^{\circ}\text{C}$ and the humidity of 40-70%, and were maintained on a commercial diet (OA-2, supplied by the Japan CLEA Co.). The composition of the diet was as follows: crude protein 26.5%, crude fat 3.5%, crude fibers 3.5%, and crude ashes 7.5% (Ca 1.8%, P 1.3%, K 0.5%, Na 0.2%). The contents of vitamin A, D, E, B₁, B₂, B₆, nicotinic acid, pantothenic acid, choline, folic acid and B₁₂ per 1 kg of the diet were 12,000 I.U., 2,400 I.U., 23 mg, 7 mg, 10 mg, 4 mg, 80 mg, 30 mg, 1,400 mg, 0.2 mg and 0.02 mg, respectively.

Pregnant rats: Two mature virgin females (supplied by the Japan CLEA Co.) were placed in a cage with one male overnight. The onset of pregnancy was considered to be the presence of vaginal plugs and spermatozoa in the vaginal smear and the day when the females showed the positive smear was regarded as zero day of gestation.

Group of animals: The experiments were performed in the following 6 groups of rats:

A) Non-pregnant rats

- 1) Group of animals fed a commercial diet alone
- 2) Group of animals fed the diet added with 10% of ethyl stearate (S-group)
- 3) Group of animals fed the diet added with 10% of ethyl linoleate (L-group)

B) Pregnant rats

- 1) Group of animals fed a commercial diet alone
- 2) Group of animals fed the diet added with 10% of ethyl stearate (PS-group)
- 3) Group of animals fed the diet added with 10% of ethyl linoleate (PL-group)

Observation: The body weight and daily food intake were observed for 45 days in the non-pregnant and pregnant rats. Observations were also performed until about 25 days after the spontaneous delivery. The level of blood glucose was estimated according to the method of Hagedorn-Jensen every 4 to 5 days. The preliminary study showed that the levels of blood glucose and tissue glycogen were considerably influenced by the time of fasting, so that following schedule of feeding was used in order to minimize the variation of glucose level. On the day before blood sampling, the animals were fasted for 3 to 4 hours from the noon and then allowed to take food for 2 hours. The animals which were fasted overnight were reallowed to take food for 2 hours in the next morning and then they were fasted for just 4 hours. At the termination, the blood sample was taken from the tail vein.

Glucose tolerance test: Glucose tolerance test was carried out in the non-pregnant rats, the pregnant rats at the 19th day of gestation, and the puerperal rats at 2 days after the delivery. They were fasted for 16 hours and then 1.5 g/kg of glucose was administered using a gastric tube. Estimation of the level of blood glucose was performed before and 0.5, 1, 2, 3 and 5 hours after the administration of glucose.

Estimation of glycogen: The non-pregnant rats at the diestrus, the pregnant rats at the 19th day, and the puerperal rats at 2 days after the delivery were guillotined under ether anesthesia, and the liver, gastrocnemius and uterus were quickly removed. Immediately thereafter, the tissues were frozen and weighed. The precipitation of tissue glycogen prepared by the modified technique of Hassid *et al.* (5) was estimated according to the anthrone method (6).

RESULTS

1) Change of the level of blood glucose during sexual cycle

The level of glucose estimated on 58 female rats showed a slight changes during

their sexual cycle. As shown in Table 1, the levels at the proestrus, oestrus and diestrus were 113.3 ± 4.4 , 110.7 ± 3.6 and 107.4 ± 4.8 mg%, respectively. The highest value was observed at the proestrus and the lowest one at the diestrus.

TABLE 1. Blood glucose level of female rats during sexual cycle.

	No. of rats	Blood glucose level (mg%)
Diestrus	11	$107.4 \pm 4.8^*$
Diestrus-Proestrus	13	112.2 ± 6.5
Proestrus	11	113.3 ± 4.4
Oestrus	23	110.7 ± 3.6

* $\pm$: Standard error

FIG. 1. Blood glucose level of the non-pregnant rats fed the standard diet alone, the diet added 10% of ethyl stearate and the diet added 10% of ethyl linoleate.
() : No. of animals.

Fig. 1 presents the influence of supplement of ethyl stearate or ethyl linoleate to the diet on the level of blood glucose in the non-pregnant rats. The supplemental procedure resulted in a slight elevation of the level during 5 to 10 days of feeding, but no significant difference was observed between the rats received the high-fat diet and the control one from the 14th to 40th day of the feeding. However, the level of blood glucose in the rats received the high-fat diet reduced slightly at the 45th day.

FIG. 2. Blood glucose level during gestation and after delivery.
() : No. of animals.

2) *Changes of the level of blood glucose during pregnancy*

As shown in Fig. 2 and Table 2, the level of blood glucose in the normal pregnant rats fed the standard diet increased slightly at the 5th day of gestation, but it decreased gradually from the 10th day. The maximum decline was attained at the 19th day of gestation and the level showed a decrease of 16.9% compared with that before the pregnancy. Immediately after the spontaneous delivery, the decreased level of blood glucose turned to increase rapidly. The level at 2 days after the delivery was 13.9% higher than that before the pregnancy. Thereafter, the level declined gradually and recovered to the control level within 10 to 26 days after the delivery.

TABLE 2. Blood glucose level during gestation and after delivery (mg%).

Days	Days after delivery	Control group (6)*	% change	PS-group (7)*	% change	PL-group (7)*	% change
Before gestation	(20)*	115.5±2.8**					
5		122.3±5.4	+ 5.9	108.0±4.7	- 6.5	108.0±5.2	- 6.5
10-11		109.9±2.8	- 9.2	100.3±6.4	-13.2	108.7±6.5	- 5.9
15		99.7±4.0	-13.7	95.0±3.2	-17.7	97.3±4.0	-15.8
19		96.0±7.4	-16.9	89.0±2.2	-22.3	95.1±5.5	-17.7
	Delivery→						
23	2	131.6±8.8	+13.9	125.7±9.3	+ 8.8	—	
25	4	—		—		118.8±6.5	+ 2.9
30-32	9-11	118.0±3.9	+ 2.2	116.3±2.4	+ 0.7	114.9±3.5	- 0.5
42	21	—		103.7±2.8	-10.2	110.6±5.5	- 4.1
45-47	24-26	105.7±3.9	- 8.5	99.0±3.0	-14.3	109.7±5.1	- 5.0

*() : No. of animals, **± : Standard error.

Daily supplement of ethyl stearate or ethyl linoleate to the diet during pregnancy modified the changes of the level of blood glucose. The elevation of the level during the early stage of gestation was not observed and the level decreased progressively in the rats received the high-fat diet. The decrease of the level at the 19th day of gestation in the rats fed the diet added with ethyl stearate and ethyl linoleate was 22.3% and 17.7%, respectively. Thus, the supplement of ethyl stearate to the diet induced the most marked decrease of the level during the late stage of gestation. The supplement of fatty acids to the diet produced a slight prevention on the hyperglycemic response which occurred immediately after the delivery. The level of blood glucose at 2 days after the delivery showed an increase of 8.8% in the rats fed the diet added with ethyl stearate and the level at 4 days after the delivery increased by 2.9% in the animals fed the diet added with ethyl linoleate.

3) *Changes in body weight and daily food intake during pregnancy*

The body weight of the control pregnant rats increased gradually and progressively, and declined markedly after the delivery. The body weight increase in the pregnant rats received the standard diet alone, the supplemental diet with ethyl stearate and the

supplemental diet with ethyl linoleate was 56, 57 and 43 g, respectively. The supplement of ethyl stearate to the diet did not influence the body weight change during pregnancy and puerperium. However, the body weight gain in the pregnant rats received the supplemental diet with ethyl linoleate was slightly less than that in the control pregnant animals. The body weight gain after the delivery was slow in both control rats and rats fed the high-fat diet. However, the gain in the latter animals was less than that in the control ones (Fig. 3).

FIG. 3. Body weight change during gestation and after delivery.
() : No. of animals.

The pregnant rats showed an increase of daily food intake during the gestation. The food intake was slightly decreased during 10 days after the delivery, and thereafter, it increased again. The reduction of the food intake during the early stage of puerperium was more marked in the rats fed the high-fat diet than in the control ones.

4) Glucose tolerance tests

As shown in Fig. 4 and Table 3, the level of blood glucose increased by 47 to 48% at 0.5 and 1 hour after the oral administration of 1.5 g/kg of glucose in the control non-pregnant rats. The level declined gradually and was almost restored to the control value at 5 hours after the administration. At the 19th day of gestation in the control pregnant rats, the elevation of the level of blood glucose was 37 to 38% at 0.5 and 1 hour after the oral administration of glucose. The increase in the pregnant rats was lower than that in the non-pregnant ones. Moreover, the level showed complete recovery at 3 hours after the oral administration.

The glucose tolerance test in the rats at 2 days after the delivery revealed that the elevation of the level of blood glucose was only 16 to 18% at 0.5 and 1 hour after the administration of glucose and it recovered to the previous level within 2 hours.

These results suggested a marked increase of glucose tolerance during the early stage of puerperium. The oral administration of glucose to the rats at 1.5 months after the

FIG. 4. Oral glucose tolerance (1.5 g/kg).

TABLE 3. Oral glucose tolerance (1.5 g/kg) during gestation and after delivery.

	Before gestation	(% change)	19th day during gestation	(% change)	2 days after delivery	(% change)	1.5 months after delivery	(% change)
No. of rats	8		3		4		4	
0	90.5±3.6*		72.0± 5.3		103.5±3.4		91.0±3.0	
0.5 hr	138.5±7.9	+47	110.0±12.2	+38	121.0±4.8	+18	122.5±3.0	+32
1 "	142.5±5.8	+48	109.3±11.1	+37	119.5±3.3	+16	128.0±2.3	+37
2 "	115.8±5.8	+25	86.6± 3.5	+15	112.5±7.6	+ 9	105.5±3.1	+15
3 "	108.5±5.5	+18	72.0± 6.4	0	110.0±3.5	+ 7	92.5±3.7	+ 2
5 "	98.3±5.1	+ 8	66.6± 4.7	-7	115.0±7.9	+13	93.0±3.0	+ 2

* ± : Standard error, Unit : mg%.

TABLE 4. Oral glucose tolerance (1.5 g/kg) in control, S- and L-group.

	Control group	(% change)	S-group	(% change)	L-group	(% change)
No. of rats	8		9		8	
0	90.5±3.6*	0	87.8±5.3	0	105.9±3.6	0
0.5 hr	138.5±7.9	+48	114.8±5.9	+26	141.5±6.8	+36
1 "	142.5±5.8	+47	120.9±4.6	+34	151.9±4.2	+46
2 "	115.8±5.8	+25	109.6±4.2	+22	119.0±4.0	+14
3 "	108.5±5.5	+18	110.0±3.8	+23	111.5±8.0	+6
5 "	98.3±5.1	+8	101.6±6.2	+14	96.5±9.6	-9

* ± : Standard error, Unit : mg%.

TABLE 5. Glycogen content in the liver, gastrocnemius and uterus during gestation and after delivery.

	No. of rats	Liver	Gastrocnemius	Uterus
Non-pregnant (Diestrus)	6	3123±631*	437±22	82±11
19th day during gestation	4	1328±279	294±76	306±69
2 days after delivery	5	1545±400	256±89	106±7

* ± : Standard error, Unit : mg/100 g of tissue.

delivery showed that the slightly increased tolerance was still remained and that the increased level of glucose at this stage was almost the same as the level at the 19th day of gestation in the pregnant rats.

The elevation of the level of blood glucose in the non-pregnant rats received the supplemental diet with ethyl linoleate for 2 months was 36 to 46% at 0.5 and 1 hour after the oral administration of glucose. The increase of the level was almost the same as that in the control non-pregnant rats, as shown in Table 4. The elevation of the level of blood glucose in the non-pregnant rats received the supplemental diet with ethyl stearate for 2 months was considerably lower than that in the control non-pregnant ones. The increase in the former rats was 26 to 34% at 0.5 and 1 hour after the administration of glucose.

5) Glycogen contents in the liver, skeletal muscle and uterus

The glycogen contents of the liver, gastrocnemius and uterus varied during pregnancy. As shown in Table 5, the glycogen contents in the liver and gastrocnemius decreased markedly, while the content in the uterus increased at the 19th day of gestation. The glycogen contents in the liver and skeletal muscle maintained still low level in 2 days after the delivery, but that in the uterus decreased markedly after the delivery.

DISCUSSION

It is well-known that pregnancy produces a variety of change in the biochemical processes of the body. In addition to the biochemical changes correlated with gonadal function, the changes in metabolic aspects of histamine (7, 8) and monoamines (9) have also been described. Tanabe (10) in this laboratory has observed that the pregnant rats are markedly susceptible to toxic manifestations of adrenocortical bleeding caused by

aminopyrine and phenylbutazone. Since the carbohydrate metabolism is closely related to the adrenocortical and ovarian function in rats, the investigation of glucose metabolism of the pregnant animals was also important to elucidate the role of endogenous steroids played in toxic manifestations of pyrazolon derivatives in the pregnant animals.

The level of blood glucose was found to decrease gradually but progressively during pregnancy until the spontaneous delivery. The decrease began from the 10th day of gestation and it was about 17% at the 19th day. Since the body weight gain of the pregnant rats was also steepened from the 10th day of gestation, the decrease in the glucose level during the late stage of pregnancy was likely to originate from the increase of glucose utilization necessary for the progressive growth of the fetuses and also from the adaptive regulation of the glucose metabolism inherent to the endocrine changes during pregnancy.

The level of blood glucose in the non-pregnant rats increased during proestrus and decreased during diestrus. Kostyo (11) and Hazelwood *et al.* (12) observed the considerable increase of glycogen contents in uterus and skeletal muscle during proestrus compared with those during diestrus in the rat. They described that the administration of estradiol to the castrated animal could elevated the tissue glycogen to the level found during proestrus phase. These findings and the evidence that the level of blood glucose increased by the administration of estrogens indicate the increased estrogenic activity during proestrus largely contributes to the increased level of blood glucose and the accumulation of glycogen in the uterus and muscle.

The glycogen content in the liver was lower during late stage of pregnancy than during non-pregnant stage, and the fasting from the 18th to 20th day of gestation did not affect the body weight gain of the fetuses (3). These findings also suggest that the hypoglycemic response during the late stage of pregnancy derives from the dominant supply of glucose to the utilization of the fetuses. In this respect, the glucose tolerance test indicated more efficient glycolysis during the late stage of pregnancy than during non-pregnant stage. The similar results were presented by Placn *et al.* (13).

It is concluded that the hyperglycemic response developed after the delivery could not be regarded only to derive from the disappearance of fetal supply of glucose, because there occurs the reduction of the daily food intake, that is, the reduction of the energy source. In addition, the increased tolerance against glucose showed that the glucose utilization was likely to be more promoted in the rats immediately after the delivery than in the non-pregnant ones. These results also indicate the increased requirement of the tissues for the glucose utilization. In the present experiments the lactation might have complicated the sequence, since the rats were allowed to rear their youngs after the delivery. The necessary prerequisite for the lactation activity such as ACTH and adrenocortical steroids may also be responsible for the elevation of the level of blood glucose after the delivery.

It has been demonstrated by several investigators that pregnancy affects markedly the fatty metabolism in the body. Boyd (1) has shown the increased level of total lipids,

triglycerides and cholesterol in the blood of pregnant rats and McKay (2) have reported the increase of total lipids in the liver as well as the decrease of depot fat during pregnancy. These findings indicate the acceleration of lipid metabolism or utilization during pregnancy and suggest more efficient and easily available mechanism of the lipid utilization than that of glucose utilization in the pregnant animals.

In the pregnant rats, the supplement of ethyl stearate or ethyl linoleate to the diet modified the changes of the level of blood glucose which observed in the control pregnant animals. Garner *et al.* (4) and Samuels *et al.* (14) have reported that the level of blood glucose and the tolerance against glucose are lower in the rats fed a high-fat diet than in the rats fed a high-carbohydrate diet. These findings suggest that utilization of lipid did not precede that of the glycogen during pregnancy.

SUMMARY

The changes of the level of blood glucose and tissue glycogen during pregnancy and after delivery were observed in the rats fed a standard diet alone and the rat fed the diet added with 10% of ethyl stearate or 10% of ethyl linoleate. Furthermore, the glucose tolerance test (oral administration of 1.5 g/kg of glucose) was performed during pregnancy and puerperium. The results obtained were as follows:

1. The level of blood glucose in the non-pregnant rats was lower at the diestrus than at the proestrus.
2. In the normal pregnant rats, the body weight increased gradually, but the level of blood glucose decreased considerably at the 19th day of gestation. The level turned to increase rapidly immediately after the spontaneous delivery. The supplement of ethyl stearate to the diet resulted in marked decrease of the level during pregnancy, and the supplement of the fatty acid produced a slight prevention of the hyperglycemic response after the delivery.
3. The elevation of the level of blood glucose induced by the glucose tolerance test was slightly lower in the pregnant rats than in the non-pregnant ones. More marked increase of glucose tolerance was observed during the early stage of puerperium.

The elevation of the level of blood glucose after the administration of glucose in the non-pregnant rats fed the diet added with ethyl linoleate was almost the same as that in the control non-pregnant ones, while the elevation in the non-pregnant rats fed the diet added with ethyl stearate was lower than that in the controls.

4. Glycogen contents in liver and gastrocnemius decreased considerably, while the content in the uterus increased markedly during pregnancy.

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