Serum Protein Binding of Exogenous Insulin in Menstruating and Pregnant Diabetic Patients

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SUMMARY

Insulin binding by insulin antibodies was determined serially in eight menstruating and in twelve pregnant diabetic subjects. No significant variation was noted in the insulin binding values before, during or after menstruation or during or after pregnancy. This constancy of insulin binding was observed despite major and minor changes in insulin needs. No causal relationship could be demonstrated between insulin binding by insulin antibodies and changing insulin requirements. If there is a relationship between the hormonal changes and the alterations in insulin needs during menstruation and pregnancy, the mechanism of this relationship is not mediated through the insulin binding capacity of circulating insulin antibodies.

Insulin-treated diabetic patients are known to develop circulating insulin antibodies.[1-7] These insulin antibodies have been demonstrated to account for the beta and gamma-globulin binding of exogenous insulin in serum.[1,2,5-7] Increased antibody binding of insulin is often associated with increased insulin requirements.[1-4] It also is known that estrogens affect binding of thyroxine and cortisol by serum protein[8,9] and that adrenal steroids affect the antibody binding of exogenous insulin.[4] Studies of the effects of various hormonal agents, such as estrogens, progesterone, growth hormone, prolactin, thyroid hormone, and adrenal steroids, in diabetes mellitus in man and animals have yielded varying results.[10-17] The mechanisms of these hormonal effects are generally unknown and are the subject of much dispute.

Menstruation and pregnancy are physiologic states that are associated with or produce hormonal changes. Alterations in the insulin requirements of patients with diabetes mellitus have been observed before, during, and after menstruation[18] and during and after pregnancy.[17,19-22] Endocrine changes have been assumed to be the cause of the altered insulin requirements. Spellacy and Goetz[23] observed fluctuations in the levels of insulin bound to gamma globulin during pregnancy. It seemed appropriate, therefore, to study antibody binding of insulin in menstruating and pregnant diabetic subjects to evaluate whether alteration of insulin binding by insulin antibodies is responsible for changes in insulin requirements during the menstrual cycle and during pregnancy.

MATERIAL AND METHODS

Eighty-nine serial specimens of serum were obtained from eight menstruating diabetic women (cases 1 to 8) and from seven pregnant diabetic patients (cases 9, 13, 15, 16, 17, 19, and 20) who were seen at the Mayo Clinic and from five pregnant diabetic patients* (cases 10, 11, 12, 14, and 18) who were seen at the University of Minnesota Medical Center. Fasting blood samples were collected during menstruation and either before or after menses, or both, in cases 1 to 8; in cases 9 to 20 they were collected during the second or third trimesters of pregnancy, or both (except in cases 16 and 17), at the time of delivery in cases 11, 13, 15, 16, 17, 19, and 20, and postpartum in all. Samples of blood were obtained every four hours on the sixth day postpartum in case 9. All specimens were allowed to clot, and the serum was promptly separated by centrifugation and was quickly frozen for storage before analysis.

A modification of the Berson-Yalow method[1] was employed. Twofold dilutions of all serum specimens were examined in duplicate by paper chromatography and undiluted specimens in cases 2 to 20 were studied also by paper electrophoresis. Radio-iodine-labeled porcine insulin (Abbott Laboratories) with a specific activity of 30 to 40 mc. per milligram of insulin (300 to 400 μc. per milliliter) was used in this study. The

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amount of damaged insulin—that is, the amount of insulin which migrated with serum proteins because of alterations produced in the insulin during the radioactive labeling process[1]—was usually less than 10 per cent but ranged as high as 15 per cent in some instances.

The serum samples were incubated at 5° C. for forty-eight hours. The chromatography and electrophoresis were carried out at room temperature (20° C.). The proportion of insulin bound to serum protein (insulin antibodies) was determined by planimetry of the peaks of radioactivity on the scanning records of the chromatograms and electrophoresis strips; the units of insulin bound per liter of serum were calculated from the amount of insulin-I-131 and of serum added. The quantity of insulin bound by insulin antibodies in the assay is an indirect measure of the insulin antibody concentration itself since the magnitude of binding (the insulin binding capacity*) is proportional to the circulating level of insulin antibodies.[7]

Specimens from cases 9, 10, 11, 13, 14, 15, 17, 18, 19, and 20 were also examined by the Skom-Talmage method.[5]† These values are expressed in terms of the percentage of insulin bound to gamma globulin (insulin antibodies).

Blood glucose determinations were carried out by the method of McGuckin[25] on samples obtained at the same time as those for insulin binding studies.

RESULTS

The binding of exogenous insulin in the specimens analyzed by electrophoresis (cases 2 to 20) occurred on proteins migrating in the inter-beta-gamma and gamma-globulin region, as has been shown by other investigators.[1,6]‡ There was no variation in this pattern in the serum samples of each subject before, during or after menstruation or during or after pregnancy.

Table 1 presents the total daily insulin doses, the fasting blood glucose concentrations, and the insulin binding values before, during and after menstruation in eight patients with diabetes mellitus. During the study, the insulin dosage* in cases 1 to 6 was relatively constant at the various times at which specimens of blood were drawn for insulin binding and fasting

TABLE 1

Daily insulin doses, fasting blood glucose concentrations, and insulin binding values in eight menstruating subjects with diabetes mellitus*

Case	Parameter†	Premenstrual	Menstrual	Postmenstrual
1	a	179	174 (2)	168
	b	73	91 (2)	243
	c	57.6	64.2 (2)	72.3
2	a	50	50	50
	b		288	73
	c		1.5	1.5
3	a	52	52	52
	b	112	129	59
	c	3.8	4.0	3.7
4	a	20	20	20
	b		153	188
	c		5.4	5.6
5	a	29 (4)	26 (2)	
	b	266 (4)	183 (2)	
	c	11.1 (4)	11.1 (2)	
6	a	36	38	36
	b	150	265	216
	c	5.5	5.3	4.5
7	a	20	15 (3)	24
	b		114 (3)	45
	c		2.2 (3)	2.1
8	a	16	5	
	b	138	127	
	c	12.7	12.5	

*Data are shown as single values or, when followed by number in parentheses, as mean of that number of observations.

†Key: a = total daily insulin dose in units per day; b = fasting blood glucose in mg. per 100 ml. of blood; and c = insulin binding values in units of insulin bound per liter of serum.

*Binding capacity implies maximal potential of the antibodies to bind both endogenous and exogenous insulin, a measurement not actually carried out in these studies. Serial additions of increasing concentrations of insulin would have been necessary to determine the true insulin binding capacity. Maximal insulin binding of the particular amount of exogenous insulin added at the given dilution of serum does occur, however, and is expressed as "insulin binding capacity" by some authors.[1,4,7,24]

†These determinations were carried out in the laboratory of Dr. F. C. Goetz, University of Minnesota Medical Center, Minneapolis, Minnesota.

‡This is believed to represent the migration pattern of insulin antibodies.[1,2,5-7]

*The insulin dosage given to these patients is assumed to be the "insulin requirement" only when attempts were made to demonstrate that this was so. Thus, in case 1 under metabolic ward conditions,[24] lowering the daily insulin dosage resulted in increased glycosuria and an increase in dosage resulted in hypoglycemic reactions. Similar observations were made in cases 5 and 7 but not in any of the others. Hence, in the remaining cases (as well as in cases 1, 5, and 7 when not under metabolic ward conditions) insulin dosage has to be interpreted as no more than a "clinical guess" at the actual need for insulin. The authors are satisfied, however, that when changes in dosage were made in these cases, these changes had sound clinical indication and are, thus, representative of altered insulin needs.

blood glucose determinations. There were hypoglycemic episodes with menstruation in cases 5 and 6, and, in cases 7 and 8, there was a decrease in insulin needs during menses.

Table 2 presents the data obtained in twelve pregnant subjects with diabetes mellitus. Thiazide drugs were used to produce diuresis in all pregnant patients for treatment of either peripheral edema, polyhydramnios, or toxemia of pregnancy during the second and third trimesters, and stilbestrol or a combination of testosterone enanthate and estradiol valerate (Deladumone) was used postpartum to prevent lactation in all cases.

The insulin requirement increased, compared to the usual dose preceding pregnancy, during the second or third trimester of pregnancy, or both, in cases 9 to 12 (20 per cent in case 12 to 109 per cent in case 11). In cases 13 and 14 the patients gave a history of increasing insulin needs during the second trimester but the serum specimens were obtained when the daily insulin dose was less than the usual dose before pregnancy. In case 15 there was no change* in insulin dosage during pregnancy, and in cases 16 to 20 there was a decrease in insulin need (from 19 per cent in case 16 to 67 per cent in case 20). The decrease in insulin requirement in case 17 appeared to be related to better dietary control of the diabetes mellitus.

On the day of delivery, as an experience-based clinical custom, the dose of insulin administered was less than the average dose before pregnancy or during the third trimester, with good control of the diabetic condition as determined by measurements of glucose in urine and blood. During the first two weeks of the postpartum period, the insulin needs stabilized at the usual prepregnancy levels in half of the cases (cases 9, 11, 13, 15, 18, and 20), at higher levels in cases 10, 12, and 19, and at lower levels in cases 14, 16, and 17.

In case 9, insulin binding was determined in specimens of blood obtained at four-hour intervals on the sixth day after delivery; the results are shown in table 3.

Table 4 presents the number of observations, the mean insulin binding value for each subject, and the standard deviations. The standard deviations were obtained by comparing the means of the observations on single specimens with the mean value of all the ob-

*A day-to-day variation of 5 per cent of the total dose is not important as a measure of changing needs; and, in fact, such a change in dosage is sometimes recommended for the purpose of gauging insulin needs.[26]

TABLE 2

Daily insulin doses, fasting blood glucose concentrations, and insulin binding values in twelve pregnant subjects with diabetes mellitus*

Case	Parameter†	Usual insulin dose (units)	During pregnancy	At delivery	Postpartum
9	a	40	73 (4)	24	47
	b		85 (4)		179
	c		9.2 (4)		9.4‡
	d		19.6 (2)		18.7‡
10	a	27	40	15	40
	b				
	c		0.1		0.1
	d		3.3		3.1
11	a	55	115	35	42.5 (8)
	b		220		254 (3)
	c		2.2	2.1	2.5 (8)
	d		9.6	4.8	4.6 (8)
12	a	40	48	16	55
	b		112		
	c		3.3		2.9
13	a	50	43 (3)	20	52 (3)
	b		39	114	178 (3)
	c		5.6 (3)	3.2	2.8 (3)
	d		8.7 (3)	7.0	5.8 (3)
14	a	60	36 (3)	25	35
	b		106		
	c		1.2 (3)		2.1
	d		6.7 (3)		6.5
15	a	30	29 (2)	10	30
	b		66	90	30
	c		0.6 (2)	0.6	0.4
	d		4.5 (2)	4.4	4.2
16	a	47	38	38	28
	b			128	44
	c			1.0	1.3
17	a	136	72	30	50
	b			50	252
	c			4.5	4.0
	d			10.4	8.1
18	a	69	48 (3)	10	48 (2)
	b		228		
	c		19.8 (3)		18.5 (2)
	d		20.1 (3)		17.2 (2)
19	a	40	35 (2)	15	35 (4)
	b			247	290 (3)
	c		9.8 (2)	10.4	9.9 (4)
	d		12.2	13.9	12.2 (2)
20	a	12	7 (4)	6	11 (2)
	b		98 (4)	62	55 (2)
	c		1.5 (4)	1.5	1.2 (2)
	d		7.8 (4)	7.5	6.9 (2)

*Data are shown as single values or, when followed by number in parentheses, as mean of that number of observations.

†Key: a = total daily insulin dose in units per day; b = fasting blood glucose in mg. per 100 ml. of blood; c = insulin binding values in units of insulin bound per liter of serum; and d = insulin binding values in terms of per cent of insulin bound to gamma globulin (Skom-Talmage method).

‡Mean of data in table 3.

TABLE 3

Serial determinations of insulin binding in serum on day six postpartum (case 9)

Time	Berson-Yalow method (U./L.*)	Skom-Talmage method (per cent†)
8 a.m.	10.8	21.0
12 noon	9.6	18.4
4 p.m.	9.6	18.8
8 p.m.	9.0	—
12 midnight	8.7	16.2
4 a.m.	8.9	19.1

*Units of insulin bound per liter of serum.
†Percentage of insulin bound to gamma globulin.

TABLE 4

Mean insulin binding values in eight menstruating and twelve pregnant diabetic subjects (Berson-Yalow method)

Patient	Number of observations*	Insulin binding value Mean ± S.D. (units/liter of serum)
Menstruating		
1	8	64.6 ± 4.28
2	6	1.5 ± 0.20
3	9	3.8 ± 0.17
4	6	5.5 ± 0.30
5	12	11.1 ± 1.23
6	6	5.1 ± 0.40
7	12	2.2 ± 0.18
8	6	12.6 ± 0.80
Pregnant		
9	20	9.3 ± 0.38
10	4	0.1 ± 0
11	20	2.4 ± 0.25
12	6	3.1 ± 0.23
13	24	3.9 ± 0.24
14	12	1.4 ± 0.13
15	8	0.5 ± 0.24
16	4	1.2 ± 0.12
17	4	4.2 ± 0.36
18	15	19.4 ± 1.22
19	14	9.9 ± 0.40
20	14	1.4 ± 0.26

*Comprises a total of eighty-nine specimens.

servations from the same subject on different days. No significant difference in the mean insulin binding value was found before, during and after menstruation or pregnancy.*

DISCUSSION

There appears to be a remarkable constancy in the binding of insulin by insulin antibodies in the same subject before, during and after menstruation and during and after pregnancy despite major and minor changes in insulin needs. This lack of direct relationship between changing insulin requirements and insulin binding values has been observed by others.[4,6,27] In addition, no correlation could be demonstrated between insulin binding levels and fasting blood glucose concentration in any of the subjects studied.

The administration of thiazides during pregnancy and of estrogens postpartum appears to have no demonstrable influence on insulin binding by insulin antibodies and could not be correlated directly with changes in insulin needs. Wolff and co-workers[28] were unable to demonstrate, in vitro, any effect of thiazide drugs on the binding of insulin to human serum protein.

The hormonal agents associated with menstruation and with pregnancy produced no consistent measurable effect on the binding of insulin by insulin antibodies. The changes in insulin requirements that are associated with menstruation and pregnancy apparently are not mediated by alterations in the circulating insulin binding capacity (of insulin antibodies). If there is a relationship between the hormonal changes and the alterations in insulin needs during menstruation and pregnancy, the basis of the mechanism is a phenomenon other than an effect on the antibody binding of insulin in the blood.

SUMMARIO IN INTERLINGUA

Ligation a Proteina Seral de Insulina Exogene in Diabeticas in Menstruation e in Pregnantia

Le ligation de insulina per anticorpore anti insulina esseva determinate serialmente in octo diabeticas in menstruation e in dece-duo diabeticas in pregnantia. Nulle significative variation esseva notate in le valores pro le ligation de insulina ante, durante, o post le menstruation e durante o post le pregnantia. Iste constantia del ligation de insulina esseva observate in despecto de major e minor alterationes in le requirimentos pro insulina. Nulle relation causal poteva esser demonstrate inter le ligation de insulina per anticorpore anti insulina e alterationes del requirimento pro insulina. Si il existe un relation inter le alterationes del requirimentos pro insulina e le alterationes hormonal durante menstruation e pregnantia, le mechanismo de iste relation non es afficite per le capacitate de ligar insulina del parte de anticorpore anti insulina in le circulation.

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