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DIURNAL VARIATION IN THE LEVELS OF GLUCOSE AND RELATED SUBSTANCES IN HEALTHY AND DIABETIC SUBJECTS DURING STARVATION

In partial fulfillment of the requirements for the degree of Master of Science.

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During an investigation of endogenous glucose turnover by isotope dilution, it was observed that whereas in healthy subjects the fasting blood glucose level remained relatively constant for several hours, in untreated maturity-onset diabetic subjects it consistently declined. Because of this absence of a steady state, it was not possible to calculate glucose turnover in the diabetic subjects. In order to determine whether more prolonged fasting would cause their blood glucose level to stabilize, two diabetic patients were followed during a forty-eight hour fast. Then it was observed that on the second morning the blood glucose levels had risen above those of the previous afternoon, and that they again fell throughout the second day. Since a diurnal [1] cycle of the blood glucose level in diabetic subjects does not seem to be generally recognized, this matter seemed worthy of further study.

An overnight rise in blood glucose levels during fasting in diabetic subjects was first observed by Hatlehol in 1924 (2). Later Mollerstrom suggested the use of this periodicity in the management of diabetes by timing insulin therapy to precede the peak in urine glucose excretion (3-6). In 1949 Izzo commented on the general lack of awareness of the diurnal rhythm in the blood glucose level in diabetes (7). He studied maturity-onset diabetic subjects during regularly spaced glucose feedings and found that the pre-feeding blood glucose levels showed a definite diurnal pattern with maximal levels in the morning. In spite of these various studies, and the

1. There is still a controversy regarding the use of the terms "diurnal" and "circadian" in describing 24-hour periodicity (1). Diurnal will be employed rather than circadian in this regard. It will not be used in the more restricted sense of describing "day-time" occurrences.

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recent interest in the general field of diurnal biorhythms (8 - 13), the endogenous periodicity of carbohydrate metabolism is not discussed in the current literature.

The aim of the present study was to obtain more complete data on the extent and timing of the diurnal variation in the blood glucose level in healthy and diabetic subjects during fasting, and to relate this variation to that of other metabolites and hormones.

SUBJECTS AND METHODS

Five healthy and five untreated maturity-onset diabetic subjects were studied. The physical characteristics of these subjects are outlined in Table 1. In all subjects a hemogram, urinalysis, blood urea nitrogen level, and liver profile were within normal limits. All the healthy subjects had a normal oral glucose tolerance test (14).

The diabetic subjects had been taking tolbutamide or chlorpropamide before the study. Two subjects, S.J. and C.K., had been on insulin therapy within the preceding year. Tolbutamide was discontinued for at least two days, and chlorpropamide for at least two weeks prior to admission to the metabolic ward. At the time of admission, the fasting blood glucose levels in the various subjects ranged from 168 to 260 mg/100 ml.

During the study the subjects were confined to the metabolic ward. For three days prior to the starvation period they were given a standard diet containing maintenance calories, of which 15 percent were derived from protein and 45 percent from carbohydrate. Then, after a 14-hour overnight fast, the three-day starvation study commenced. Only water, black tea or coffee were given during this time. Thirty ml of venous blood were taken at 8 a.m. on the first day, and every four hours thereafter. Four-hour voided urine specimens were also collected to coincide with the blood samples.

The blood samples for glucose analysis were stored overnight at 4 °C. The serum, plasma and urine samples were stored at -4 °C for later analysis.

Levels of blood and urine glucose, serum and urine inorganic phosphate, and urine urea nitrogen were measured by means of a Technicon Autoanalyzer. Plasma cortisol levels were measured by the method of Murphy and Pattee (15). Serum insulin levels were measured by the radioimmunoassay of Morgan and Lazarow (16). Serum free fatty acid levels were measured by the method of Dole (17). Serum and urine ketone levels were measured by the method of Nadeau, which detects acetoacetate plus acetone (18). Serum triglyceride levels were measured by the method of van Handel and Zilversmit (19).

Urine urea nitrogen excretion was used as an index of protein catabolism. When compared on the urine samples from one subject, its ratio to the total nitrogen excretion, as measured by the micro-Kjeldahl method (20), was 0.86 ± SD 0.05 on the first day and 0.71 ± SD 0.04 on the third day (21).

Serum insulin levels in subjects S.J. and C.K., who had been on recent insulin therapy, were greatly elevated apparently due to interference with the immunoassay by insulin antibodies in the subjects' sera (22), and are not presented.

RESULTS

All the measurements on the four-hour blood and urine samples from the individual healthy and diabetic subjects during the three-day starvation period are presented in Tables 2 and 3.

The levels of each moiety were examined for day to day changes and for within day changes by analysis of variance (23). Probability values for those changes which are statistically significant at the 5 percent level are presented in Table 4.

Day to day variation was assessed by comparing Day 1 levels with the mean of Day 2 and Day 3 levels, and Day 2 levels with Day 3 levels. The results of this analysis appear on the left side of Table 4. Within day variation was assessed by analyzing the combined data from the three days for significant quadratic and linear components. The results of this analysis are on the right side of Table 4. A quadratic component is one with a single centre of curvature, and therefore its presence within 24-hour periods indicates a diurnal cycle. A linear component usually resulted from a steady rise or fall throughout the 72-hour period, but in this case it was accompanied by significant day to day variation, and does not indicate a diurnal cycle. In the absence of day to day variation, a significant linear component does indicate a diurnal cycle. This occurred only in the case of urea nitrogen excretion in the diabetic subjects.

Individual and mean blood glucose levels from the healthy and diabetic subjects are presented in Figure 1 [2]. In the healthy subjects the mean blood glucose level declined during the first day from 87 to 64 mg/100 ml and then remained stable. No

2. The probability values for the day to day changes and the within day changes in all the figures are presented in Table 4, and are therefore omitted from the text.

significant diurnal cycle was present. In the diabetic subjects the mean blood glucose level fell throughout the three-day period from 191 to 115 mg/100 ml. In three of these subjects the blood glucose levels were within or near the normal "fasting" range by the afternoon of the second day. Also in the diabetic group, a significant diurnal cycle was superimposed on this general decline in the blood glucose level, with peak levels occurring near 8 a.m. This cycle was best observed in the subject with the highest initial blood glucose level. In all subjects the amplitude of the cycle diminished during successive days as the blood glucose level fell. This correlation between amplitude and level ($r = 0.85$, $p < 0.01$) has also been described for other diurnal biorhythms (24, 25).

This amplitude-level relationship led Sollberger (26) to describe methods for the conversion of diurnal biorhythm data into relative values, in order to make cycles occurring at different levels more comparable. The relative value is the ratio of the absolute level to the base-line level. The blood glucose data in the present study were converted to relative values, the base-line being derived by the method of moving means (26). The average relative values for the healthy and the diabetic subjects are shown in Figure 2. They demonstrate more clearly the diurnal cycle in the diabetic subjects, and the random variation in the healthy subjects, than do the absolute values plotted in Figure 1.

Glycosuria did not occur in the healthy subjects and in three of the diabetic subjects. The other two diabetic subjects had only traces of glucose in the urine.

Mean plasma cortisol levels from the healthy and the diabetic subjects are presented in Figure 3. In the healthy subjects the mean initial cortisol level was 10 ug/100 ml. In the diabetic subjects the mean initial level was 20 ug/100 ml, which is significantly higher than that of the healthy subjects ($t=2.64$, $p < 0.05$). The mean plasma cortisol levels rose during starvation in both groups, but remained significantly higher in the diabetic group throughout the three-day period. (sign test for differences between means, $p < 0.001$). The degree of rise was not different in the two groups ($t = 0.14$, $p > 0.8$). By the morning of the fourth day the mean levels were 19 ug/100 ml in the healthy subjects and 30 ug/100 ml in the diabetic subjects. Superimposed on the general rise in mean plasma cortisol levels there was in both groups a marked diurnal cycle, with peak levels at 8 a.m.

Serum insulin levels in the healthy subjects, and in the diabetic subjects who had not received insulin within the previous year, are shown in Figure 4. These levels, especially in the healthy group, fluctuated in an apparently random manner. There were no significant day to day changes or within day changes in either group. The serum insulin levels were within much the same range in all the subjects, with the exception of those in the non-diabetic subject K.Y., which were distinctly higher. This was presumably related to her obesity (27).

Mean serum and urine inorganic phosphate levels, and urine urea nitrogen levels, are presented in Figure 5. In both the healthy and the diabetic subjects the serum and urine phosphate levels were higher during the second and third days that during the first day. In the healthy subjects significant diurnal cycles were not present. In the diabetic subjects diurnal cycles

were present in both the serum and urine levels. The peak serum phosphate levels occurred near 4 a.m. The urine phosphate levels varied reciprocally with the serum levels. The urea nitrogen excretion did not show significant day to day variation in either the healthy or the diabetic subjects. A significant diurnal cycle was not present in the healthy subjects, but was present in the diabetic subjects, with higher values during the day-time period.

Individual and mean serum free fatty acid levels are presented in Figure 6. In the healthy subjects the mean level rose during the study from 0.7 to 1.8 mEq/l, and in the diabetic subjects from 1.0 to 1.7 mEq/l. The initial level in the diabetic subjects was higher ($t = 2.30$, $p = 0.05$), whereas the mean rise was lower ($t = 2.35$, $p < 0.05$), than in the healthy subjects. A diurnal cycle was not present in either group. It may be noted that there was little, if any, rise in the serum free fatty acid levels in the overweight diabetic subjects S.J. and C.K. during starvation, and that the rise in the overweight non-diabetic subject K.Y. was also somewhat less than that of the other members of her group.

Individual and mean serum ketone levels are presented in Figure 7. In the healthy subjects the mean level rose from 0.9 to 7.4 mg/100 ml, and in the diabetic subjects from 0.9 to 13.2 mg/100 ml. The initial levels were the same in the two groups, and the increments were not significantly different ($t = 1.54$, $p > 0.1$). However, within the diabetic group the response to starvation in the two slender men was distinctly greater than in the three overweight women. A significant diurnal

cycle in the serum ketone levels was not present in either group. This may be because of the very low levels near the beginning of the study. Inspection of the data from the third day in the diabetic subjects suggests that a cycle was beginning to emerge.

Mean urine ketone excretion is presented in Figure 8. The mean excretion rose during the starvation period in both the healthy and the diabetic subjects. A significant diurnal cycle was not present in the healthy subjects. A well marked diurnal cycle was present in the diabetic subjects, with peak values between 4 and 8 a.m.

Mean serum triglyceride levels are presented in Figure 9. In the healthy subjects the mean level fell significantly from 127 to 94 mg/100 ml by the evening of the second day, but rose to near its initial value on the third day. A diurnal cycle was not present. In the diabetic subjects the mean level fell during the three-day period from an initial value of 151 mg/100 ml, which is not significantly different from that of the healthy subjects ($t = 1.12$, $p > 0.2$), to a final value of 114 mg/100 ml. A significant diurnal cycle was present, with maximal levels between 4 and 8 a.m.

A summary of the findings during the three-day starvation period in the healthy and the diabetic subjects is presented in Table 5.

DISCUSSION

A diurnal cycle was superimposed upon the overall decline in the blood glucose level in untreated maturity-onset diabetic subjects during three days of starvation. The peak blood glucose levels occurred near 8 a.m. The amplitude of the cycle was proportional to the blood glucose level. These findings confirm and extend previous observations by Hatlehol (2) and Hopmann (28) in fasting subjects, and by Mollerstrom (3-6) and Izzo (7) in fed subjects. The diurnal

cycle does not appear to be related to food or activity, for it persists not only during total starvation, but also during alterations in the feeding pattern (7), and complete bed rest (2).

The presence of a diurnal cycle of the blood glucose level in health has been controversial (11). It was not demonstrated in the present study. The failure to do so may reflect the observed correlation between amplitude and level. The amplitude of the cycle at normoglycemic levels may be so small that it is not statistically detectable in small groups by the present methods.

The diurnal cycle of the blood glucose level in the diabetic subjects in the present study is in phase with an hepatic glycogen cycle which has been observed in several species (25, 29). This glycogen cycle also persists during starvation. An attempt to demonstrate an hepatic glycogen cycle in man by means of liver biopsies (30) was inconclusive. Studies of human liver temperature suggest that such a cycle may be present (31). The minimal temperature in the early morning may reflect the endothermic reactions of hepatic glycogen storage.

Urine nitrogen excretion in rabbits parallels their hepatic glycogen content (29). Similarly, the urea nitrogen excretion in the present diabetic subjects paralleled their blood glucose level. It seems unlikely that this urea nitrogen cycle was related to diurnal changes in renal function, for not only was it out of phase with glomerular filtration (1), but also it did not occur in the healthy subjects. Therefore the relationships between the blood glucose, hepatic glycogen, and urine nitrogen cycles in the various species suggest that the peak in the blood glucose cycle in the present diabetic subjects was related to enhanced hapetic gluconeogenesis in the early morning.

Alterations in peripheral glucose utilization may also have been a factor in producing the blood glucose cycle in the diabetic subjects. This is suggested by the coincidence of the peaks in their serum phosphate and blood glucose levels, although it should be noted that phosphate excretion was minimal at these times. The absence of the cycle in the serum free fatty acid levels, which would be expected to accompany changing peripheral glucose utilization, may have been due to such factors as the large differences in levels among individuals, the lability of the serum free fatty acid level (32), and the buffering effect of the tissue free fatty acid reservoir (33). That there was in fact a diurnal cycle in free fatty acid release from adipose tissue in the diabetic subjects, in phase with that of the blood glucose cycle, is suggested by the rhythmicity of the urine ketone excretion and the serum triglyceride levels in this group. The peak ketone and triglyceride levels occurred between 4 and 8 a.m., suggesting their enhanced hepatic production from serum free fatty acids at this time. The presence of a ketone cycle in diabetes has previously been observed (6).

Thus, there is evidence to suggest that the diurnal cycle in the blood glucose level in diabetic subjects may be due to rhythmic alterations in both hepatic glucose production and in peripheral glucose utilization. Although the cause of these changes is unknown, it is reasonable to speculate that they may be related to the cycle in the plasma cortisol level (34). The dual role of cortisol in stimulating hepatic gluconeogenesis (35, 36) and in impairing peripheral glucose uptake (37 - 39) is well established. The plasma cortisol rhythm persisted during starvation, confirming previous studies in mice (40). The blood glucose and plasma cortisol cycles coincided in time, with peak levels near 8 a.m. The alteration of the liver

glycogen cycle in chickens by light-dark reversal (25) is consistent with its regulation by plasma cortisol under the cyclic influence of adrenocorticotropin (34, 41, 42). Unfortunately, direct evidence from studies of the liver glycogen cycle in adrenalectomized animals is conflicting (8, 25). In order further to examine the relationship between the blood glucose and the plasma cortisol cycles in man, studies could be carried out in diabetic subjects to see whether the blood glucose cycle is altered by light-dark reversal (43), and whether it persists following total blindness (44), and adrenocortical insufficiency or hyperfunction (34).

It is noteworthy that while the plasma cortisol cycle in the diabetic subjects was similar to that of the healthy subjects, the plasma cortisol level in the diabetic group was higher throughout. This confirms other recent reports of elevated corticosteroid levels in both the blood and urine in diabetes (45-47). These findings suggest that cortisol production is increased in diabetes. The role played by diminished detoxification of cortisol (48) is unclear, for in liver disease, where detoxification is impaired, serum corticosteroid levels are not elevated and urine levels are low (49). Elevation of the plasma cortisol levels was not related to obesity in this study, and it has been shown previously that despite increased corticosteroid excretion in obesity, plasma levels are normal or low (50, 51). Thus, the available evidence suggests that functional adrenocortical hyperplasia may be one of the components of the diabetic state.

It seemed likely that the absence of a significant diurnal cycle in glucose and related metabolites in the healthy subjects was due to damping of the glucose cycle by the secretion of insulin. In the diabetic subjects, on the other hand, it seemed probable that

an ineffectual insulin response allowed the cycles to become manifest. However, cyclic changes in the insulin levels in peripheral venous blood were not demonstrated in the healthy subjects. This may have been due to trapping of endogenous portal venous insulin by the liver (52, 53), or it may indicate that insulin is not the major factor which governs this aspect of blood glucose homeostasis.

In addition to diurnal rhythmicity, some other metabolic aspects of starvation appear in the present study. The decline in the fasting blood glucose level was presumably related to decreased hepatic glycogen reserves. Its extent in the diabetic subjects seems noteworthy, and recalls the therapeutic use of periodic fasts in the pre-insulin era (54). The absence of a change in the serum insulin level upon carbohydrate withdrawl, despite lowering of the pancreatic insulin content (55), confirms all but one (56) of previous studies (57-59). The serum and urine phosphate levels increased during the three days of starvation, while the urine urea nitrogen level did not change. The response of these levels in other studies of short starvation periods has been variable (21, 57, 60), and probably reflects the preceeding intake.

The rise in the serum cortisol level during starvation confirms previous observations in man (60, 61), and in mice (40). It may be due to impaired cortisol detoxification, since urine corticosteroid levels concomitantly diminish (60). The increased serum cortisol level in starvation may play an adaptive role by enhancing gluconeogenesis.

The presence of an elevated serum free fatty acid level in diabetic subjects after an overnight fast is well known (62). Slight elevation of the fasting serum total ketone level has also been observed (63). The absence in the present study of elevated serum acetoacetate and acetone levels is consistent with the

observation that beta-hydroxybutyrate may be the most labile of the serum ketone bodies (64). The response of the serum free fatty acid and serum ketone levels to starvation was less in the overweight subjects, particularly in those who were diabetic, than in the slender subjects. It did not appear to be related to sex (65). The influence of obesity upon this response has previously been reported in non-diabetic subjects (66 - 68), but comparative studies in diabetic subjects appear to be lacking. It seems likely to us that the lesser response in obesity is not due to diminished free fatty acid mobilization, as has previously been suggested (66 - 68), but rather to increased utilization to meet the greater caloric demands of the obese state.

The serum triglyceride levels in the healthy subjects showed a biphasic response, falling first, and then rising to near their initial level. This may reflect delay in conversion to amino acids and glycerol instead of glucose as a source for hepatic alpha-glycerol phosphate during starvation. In the diabetic subjects the initially elevated serum triglyceride level steadily fell during the starvation period, perhaps reflecting the subsidence of carbohydrate-induced hyperlipemia in this group (69, 70). Previous observations (71 - 73) on the response of the serum triglyceride levels to starvation are inconclusive.

Finally, it may be recalled that the present study was undertaken after an attempt to measure endogenous glucose turnover in diabetic subjects by isotope dilution had been unsuccessful because of the cyclic changes in their blood glucose levels. This absence of a steady state may account in part for the glucose turnover in diabetes in man being variously reported as diminished (74, 75) normal (76, 77), and increased (78, 79). Since the measurement of

of glucose turnover in the non-steady state by isotope dilution poses a number of problems, the resolution of these differences may be difficult.

SUMMARY

A diurnal cycle in the blood glucose level was observed in five maturity-onset diabetic subjects during three days of starvation. The peak blood glucose levels occurred near 8 a.m. The amplitude of the cycle was proportional to the blood glucose level. A diurnal cycle was not demonstrated in five healthy subjects.

The diurnal cycle of the blood glucose level in the diabetic subjects correlated with similar cycles in urine urea nitrogen excretion, the serum phosphate level, urine ketone excretion, the serum triglyceride level, and the plasma cortisol level. Only the plasma cortisol cycle was observed in the healthy subjects. These cycles were in phase with an hepatic glycogen cycle which has previously been observed in several animal species. These relationships suggest that the blood glucose cycle in the diabetic subjects is due to rhythmic alterations in both hepatic glucose production and peripheral glucose utilization, perhaps resulting from cyclic changes in the plasma cortisol level. The absence of these effects of cortisol in health could be due to damping by the secretion of endogenous insulin.

The initial fasting plasma cortisol level was higher in the diabetic than in the healthy subjects. In both groups the level rose during the starvation period, but remained distinctly higher in the diabetic group throughout. Consideration of the present and previous evidence suggests that functional adrenocortical hyperplasia may be one of the components of the diabetic state.

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TABLE 1

PHYSICAL CHARACTERISTICS OF SUBJECTS

Group	Subject	Sex	Age	Height (Inches)	Weight (Pounds)	Weight (% of ideal)
HEALTHY	1. F.J.	M	63	70	155	96
	2. S.S.G.	M	59	63	140	103
	3. E.P.	M	57	72	173	101
	4. E.L.	F	51	64	131	100
	5. K.Y.	F	60	64	208	158
DIABETIC	1. S.W.	M	53	62	131	100
	2. W.D.	M	71	67	161	106
	3. S.J.	F	70	60	137	118
	4. R. A.	F	47	65	202	149
	5. C. K.	F	59	60	206	174

TABLE 2a - Blood levels during the three-day starvation period in healthy subjects.

		Day 1						Day 2						Day 3						
Moiety	Subject	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.
Blood Glucose (mg/100 ml)	F. J.	80	73	67	64	58	59	57	61	64	62	57	62	58	58	75	63	61	59	58
	S. S. G.	89	80	65	73	70	65	57	60	62	61	63	63	57	51	52	57	51	53	55
	E. P.	99	85	75	71	76	74	69	71	65	71	70	65	70	75	73	67	67	68	72
	E. L.	84	73	75	69	75	69	61	62	63	59	67	66	68	67	69	63	65	73	70
	K. Y.	85	79	81	76	83	83	77	79	81	82	79	75	73	67	72	76	71	79	65
	Mean	87	78	73	71	72	70	64	67	67	67	67	66	65	64	68	65	63	66	64
Plasma Cortisol (ug/100 ml)	F. J.	13	13	6	3	5	9	14	9	17	3	4	18	11	14	11	6	11	11	20
	S. S. G.	13	13	7	11	15	15	29	20	20	5	19	14	8	13	9	10	8	16	9
	E. P.	9	5	7	2	0	2	9	5	3	0	5	3	16	3	4	1	6	8	7
	E. L.	6	7	11	5	4	13	15	11	6	11	11	17	17	12	15	10	12	20	25
	K. Y.	10	9	5	9	7	15	13	12	8	9	12	11	23	14	16	21	16	23	32
	Mean	10	9	7	6	6	11	16	11	11	6	10	13	15	11	11	10	11	16	19
Serum insulin (μU/ml)	F. J.	15	27	27	28	24	13	12	18	22	15	24	12	6	15	15	8	24	24	27
	S. S. G.	68	70	49	75	95	46	51	55	52	74	75	93	64	93	50	47	53	46	54
	E. P.	32	38	22	24	13	22	13	13	6	9	47	12	17	27	24	3	13	22	16
	E. L.	5	4	4	5	6	4	4	3	2	3	5	4	3	3	3	3	8	23	4
	K. Y.	101	88	116	82	122	130	146	121	136	112	112	146	115	119	143	147	168	132	118
	Mean	44	45	44	43	52	43	45	42	44	43	53	53	41	51	47	42	53	49	44
Serum phosphate (mg/100 ml)	F. J.	3.0	3.2	3.1	3.1	3.3	2.7	2.9	3.2	2.9	3.0	3.0	2.8	3.1	3.1	3.4	3.1	3.1	3.0	3.0
	S. S. G.	3.0	3.4	3.6	3.0	3.0	3.7	3.3	3.8	3.4	3.3	3.2	3.2	3.2	3.4	3.5	3.4	3.3	2.7	3.5
	E. P.	2.7	3.2	3.3	2.3	3.5	3.5	3.3	3.4	3.5	3.4	3.4	3.5	3.2	3.5	3.4	3.4	3.0	3.2	3.3
	E. L.	3.1	3.2	3.2	3.2	3.6	3.8	3.5	3.7	3.7	3.7	3.8	4.1	3.8	3.5	3.4	3.4	3.6	3.3	3.6
	K. Y.	3.4	3.3	3.3	3.3	3.4	3.8	3.5	3.2	3.4	3.5	3.5	3.7	3.8	3.7	3.7	3.5	3.9	3.8	3.7
	Mean	3.0	3.3	3.3	3.0	3.4	3.5	3.3	3.5	3.4	3.4	3.4	3.5	3.4	3.4	3.5	3.4	3.4	3.2	3.4

TABLE 2a -- Continued.

		Day 1						Day 2						Day 3						
Moiety	Sub-ject	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.
Serum free fatty acids (mEq/l)	F.J.	0.65	1.00	1.14	1.20	1.40	1.35	1.57	1.46	1.60	1.16	1.15	1.88	1.42	2.31	1.08	1.54	1.60	1.59	1.65
	S.S.G.	0.76	1.17	1.28	1.60	1.36	1.92	1.25	1.38	1.68	2.03	2.33	1.87	2.14	1.89	1.93	1.75	1.54	1.69	1.75
	E.P.	0.38	0.58	0.76	1.00	0.85	0.74	0.98	1.11	1.23	1.19	1.29	1.26	1.89	1.81	2.30	1.56	1.31	1.18	1.19
	E.L.	0.65	0.86	1.51	1.64	1.96	1.80	1.70	1.86	1.70	1.99	1.71	1.35	2.36	2.10	1.86	2.10	1.91	1.99	2.23
	K.Y.	1.12	1.32	1.35	1.37	1.32	1.10	1.28	1.44	1.49	1.71	1.52	1.59	1.42	1.24	1.61	1.88	1.47	1.26	1.99
	Mean	0.71	0.99	1.21	1.36	1.38	1.38	1.36	1.45	1.54	1.62	1.60	1.59	1.85	1.87	1.76	1.77	1.57	1.54	1.76
Serum ke-tones (mg/ 100 ml)	F.J.	0.8	2.3	1.2	2.3	3.0	4.0	2.9	3.0	3.8	3.8	3.9	4.6	5.2	5.3	6.2	4.8	4.7	5.6	7.1
	S.S.G.	1.1	0.9	2.2	2.1	2.5	3.1	4.0	4.8	6.8	6.9	6.6	5.9	5.5	6.0	3.5	10.4	10.5	9.3	9.8
	E.P.	1.7	0.6	1.7	1.7	2.7	2.0	4.0	3.3	4.4	4.6	4.8	5.0	4.0	2.9	3.5	4.5	6.3	6.7	6.2
	E.L.	0.0	0.0	0.0	0.7	0.9	1.4	1.6	1.5	2.9	3.3	4.3	3.7	3.6	3.7	4.8	6.1	5.0	6.2	5.1
	K.Y.	0.8	1.1	0.7	1.5	2.4	1.1	2.1	2.5	4.1	2.2	3.8	6.2	4.5	6.5	5.2	8.0	6.8	5.5	8.6
	Mean	0.9	1.0	1.2	1.7	2.3	2.3	2.9	3.0	4.4	4.2	4.7	5.1	4.6	4.9	4.6	6.8	6.7	6.7	7.4
Serum trygly-cerides (mg/ 100 ml)	F.J.	108	115	98	112	98	105	113	95	114	116	126	137	152	159	162	178	169	174	171
	S.S.G.	105	104	110	101	85	74	61	93	81	56	37	42	68	67	65	70	77	92	77
	E.P.	120	128	124	117	113	137	120	107	120	96	148	131	151	138	133	146	144	173	146
	E.L.	129	127	122	125	124	198	127	116	98	80	106	114	102	106	106	120	124	112	131
	K.Y.	172	135	170	156	150	161	166	138	135	122	122	116	124	115	107	104	111	133	119
	Mean	127	122	125	122	114	135	117	110	110	94	108	108	119	117	115	124	125	137	129

TABLE 2b -- Urine levels during the three-day starvation period in healthy subjects.

		Day 1						Day 2						Day 3					
Moiety	Subject	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.
Urine phosphate (mg/4 hr)	F.J.	81	143	255	181	124	80	44	167	115	185	386	134	166	143	136	7	113	101
	S.S.G.	86	110	110	67	66	132	54	127	184	127	67	314	177	98	114	305	87	105
	E.P.	65	74	112	114	37	56	33	166	145	117	59	69	102	95	315	78	102	54
	E.L.	79	119	84	91	104	118	125	166	190	143	160	148	181	190	162	150	158	152
	K.Y.	40	61	223	28	120	66	32	19	46	101	74	327	39	78	198	48	278	126
	Mean	70	102	157	96	90	90	58	129	136	134	149	198	133	121	185	118	148	108
Urine urea nitrogen (mg/4 hr)	F.J.	1090	1950	4250	2860	2200	1210	750	2370	2500	3200	4890	1680	1590	1320	1610	340	1630	1804
	S.S.G.	1780	1370	1300	570	1040	880	1360	1420	1070	930	2170	1130	1050	840	420	1220	1170	1094
	E.P.	2320	1490	1900	1590	1580	1010	700	2070	2310	2030	960	1790	2210	2210	2500	1430	1580	1164
	E.L.	1680	1230	1420	650	1040	1140	1120	1660	1720	1360	1680	1390	1530	1900	660	1330	820	1558
	K.Y.	1360	730	1340	1240	1100	930	960	1140	1140	1170	1200	1110	910	1510	960	1000	1330	1170
	Mean	1650	1350	2040	1380	1390	1030	980	1730	1750	1740	2180	1420	1460	1560	1230	1060	1310	1360
Urine Ketones (mg/4 hr)	F.J.	0.0	9.6	21.3	16.4	32.8	10.4	10.3	47.5	41.8	34.3	95.3	27.6	44.4	28.7	34.1	4.2	37.0	9.2
	S.S.G.	0.0	0.0	0.0	0.6	2.1	40.6	8.2	26.0	22.5	6.4	27.0	77.1	38.4	44.2	49.3	5.7	57.8	59.0
	E.P.	12.0	0.0	0.0	3.2	3.6	4.2	4.6	22.3	26.3	26.8	1.7	17.4	28.5	18.5	21.3	19.5	32.2	19.4
	E.L.	0.0	3.2	5.2	8.5	7.9	16.3	20.8	23.5	30.2	21.0	36.1	25.1	35.4	47.8	48.6	51.3	27.8	38.2
	K.Y.	0.0	0.0	7.1	8.3	4.7	4.5	7.7	21.5	35.4	32.2	29.9	48.8	88.5	70.2	57.4	33.5	82.7	51.8
	Mean	2.4	2.6	6.7	7.4	10.2	15.2	10.3	28.2	31.3	24.1	38.0	39.2	47.0	41.9	42.1	22.8	47.5	35.5

TABLE 3a - Blood levels during the three-day starvation period in diabetic subjects.

		Day 1						Day 2						Day 3						
Moiety	Subject	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.
Blood Glucose (mg/100 ml)	S.W.	144	136	123	110	106	94	103	97	101	87	77	73	81	100	105	106	104	100	110
	W.D.	189	167	139	126	121	124	125	114	109	107	95	93	100	118	114	116	115	111	123
	S.J.	158	142	124	137	135	126	127	120	114	114	115	111	111	104	86	95	92	86	82
	R.A.	189	152	123	120	110	115	137	125	107	106	101	97	88	100	86	84	79	73	80
	C.K.	276	234	190	160	178	190	218	202	152	172	198	184	178	180	154	154	168	178	182
	Mean	191	166	140	131	130	130	142	132	117	117	117	112	112	120	109	111	112	110	115
Plasma cortisol (ug/100 ml)	S.W.	20	23	17	11	11	23	14	11	8	5	8	20	17	20	7	11	17	21	26
	W.D.	17	15	12	12	11	14	14	21	21	9	25	19	27	17	21	15	15	34	32
	S.J.	25	16	13	20	18	25	25	20	24	18	22	24	21	25	17	26	23	19	23
	R.A.	9	5	5	2	9	13	5	13	3	15	10	15	18	15	19	17	19	29	44
	C.K.	30	20	15	11	24	17	34	18	20	15	13	16	23	19	16	15	16	21	24
	Mean	20	16	12	11	15	18	18	17	13	12	16	19	21	19	16	17	18	25	30
Serum insulin (μU/ml)	S.W.	33	34	37	35	55	25	28	29	27	33	20	24	23	29	24	25	16	41	38
	W.D.	43	24	13	26	29	25	43	24	40	32	22	14	23	32	30	12	21	30	55
	R.A.	18	16	19	14	20	18	16	19	14	19	6	13	11	10	15	14	12	11	17
	Mean	31	25	23	25	35	23	29	24	27	28	16	17	19	23	23	17	16	27	37
Serum phosphate (mg/100 ml)	S.W.	3.0	3.1	3.1	3.4	3.3	3.6	3.5	3.5	3.4	3.5	3.7	3.8	3.5	3.9	3.6	3.7	3.4	3.7	3.4
	W.D.	2.7	2.7	3.0	3.0	3.0	2.9	3.0	3.0	3.0	3.2	3.1	3.2	3.1	3.0	3.2	3.2	3.0	3.0	3.1
	S.J.	3.4	3.0	3.6	3.4	3.6	3.8	3.8	3.8	3.8	3.6	3.8	4.0	4.2	3.8	3.8	3.5	3.5	4.0	4.0
	R.A.	3.7	3.7	3.8	3.9	4.1	4.5	4.4	4.3	4.4	4.0	4.3	4.2	4.3	4.2	4.1	3.9	4.1	4.2	4.1
	C.K.	2.9	3.1	3.1	3.0	3.4	3.6	3.6	3.6	3.3	3.3	3.5	3.5	3.5	3.0	3.4	3.3	3.3	3.6	3.4
	Mean	3.1	3.1	3.3	3.3	3.5	3.7	3.7	3.6	3.6	3.5	3.7	3.7	3.7	3.6	3.6	3.5	3.5	3.7	3.6

TABLE 3a -- Continued.

		Day 1						Day 2						Day 3						
Moiety	Sub-ject	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.	12 n.	4 pm.	8 pm.	12 mn.	4 am.	8 am.
Serum	S.W.	0.82	1.16	1.63	1.50	1.09	1.39	2.12	1.80	1.66	1.66	1.51	1.55	2.04	1.94	2.06	1.61	1.10	1.59	2.1
free	W.D.	1.09	1.45	1.21	1.45	1.32	1.83	1.43	1.42	1.30	1.54	2.56	1.76	1.81	1.39	1.62	1.83	1.53	1.45	2.2
fatty	S.J.	0.82	0.72	0.95	1.09	1.05	0.79	0.98	1.07	1.23	1.31	1.11	0.91	0.93	1.05	1.04	1.36	1.10	0.99	1.2
acids	R.A.	1.51	1.48	1.25	1.46	1.73	1.02	1.38	1.71	1.40	1.44	1.40	1.34	1.53	1.24	1.74	1.47	2.56	1.46	1.9
(mEq/l)	C.K.	0.84	0.86	0.76	0.81	0.89	0.70	0.86	0.74	0.77	0.78	0.75	0.92	0.75	0.97	0.83	0.85	1.07	0.85	0.8
	Mean	1.02	1.13	1.16	1.26	1.22	1.15	1.35	1.35	1.17	1.35	1.47	1.30	1.41	1.32	1.46	1.42	1.47	1.27	1.6
Serum	S.W.	1.4	2.2	2.5	3.7	4.1	3.1	5.6	7.3	9.2	9.4	11.8	12.3	15.1	10.7	10.7	15.1	13.4	18.0	24.5
ke-	W.D.	1.0	1.8	1.4	2.9	3.3	3.8	3.4	5.1	6.0	5.0	5.7	10.9	11.1	10.7	11.4	15.3	21.3	20.0	19.2
tones	S.J.	0.6	0.8	1.2	1.0	1.6	1.1	0.8	2.3	2.2	1.9	1.8	3.2	3.6	3.0	2.9	4.1	4.3	4.4	4.5
(mg/	R.A.	1.0	1.0	1.0	0.9	1.2	1.9	2.0	1.5	2.3	3.3	4.2	4.0	6.4	5.0	6.6	6.5	7.6	8.9	9.5
100 ml)	C.K.	0.3	1.1	0.9	1.2	2.0	1.8	3.4	2.9	2.8	4.1	6.3	2.8	5.0	4.5	3.0	5.1	8.6	7.5	8.1
	Mean	0.9	1.4	1.4	1.9	2.4	2.3	3.0	3.8	4.5	4.7	6.0	6.6	8.2	6.8	6.9	9.2	11.0	11.8	13.2
Serum	S.W.	83	84	99	91	109	132	60	63	64	71	75	60	75	44	46	57	61	61	65
trygly-	W.D.	141	120	110	96	95	124	123	108	72	72	68	82	77	70	66	71	77	72	87
cerides	S.J.	251	229	233	212	214	274	223	207	192	211	198	176	171	169	119	152	163	177	186
(mg/	R.A.	122	109	137	111	128	116	122	130	94	96	147	100	115	102	95	108	70	112	110
100 ml)	C.K.	159	164	166	151	174	189	189	166	142	139	141	130	148	114	133	131	120	128	139
	Mean	151	141	149	132	144	167	143	135	113	118	126	110	117	100	92	104	98	110	117

TABLE 3b -- Urine levels during the three-day starvation period in diabetic subjects.

		Day 1						Day 2						Day 3					
Moiety	Subject	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.	8-12 am.	12-4 pm.	4-8 pm.	8-12 pm.	12-4 am.	4-8 am.
Urine phosphate (mg/4 hr)	S.W.	91	52	90	218	100	106	140	169	171	150	113	118	136	211	106	278	143	110
	W.D.	123	95	185	113	112	105	160	167	131	143	147	166	233	213	238	215	171	168
	S.J.	77	180	43	74	111	79	100	133	135	121	115	102	142	160	144	149	94	134
	R.A.	123	127	137	107	118	24	107	237	281	275	247	58	272	121	294	91	198	150
	C.K.	90	120	36	73	73	14	17	131	96	49	32	29	24	33	43	99	57	50
	Mean	101	115	98	117	103	66	105	167	163	148	131	95	161	148	165	166	133	122
Urine urea nitrogen (mg/4 hr)	S.W.	1170	700	370	210	890	1010	1630	1470	1400	780	910	1020	1210	860	620	2280	930	760
	W.D.	1400	1080	1690	1100	1020	680	1460	1620	1010	1020	960	880	1320	1790	2260	1290	980	890
	S.J.	2530	2930	1300	1220	1940	900	980	1300	1280	1770	1320	800	1000	1170	1000	1030	660	1030
	R.A.	1840	1520	1430	1380	920	870	1480	1680	1540	1040	930	750	1630	1570	1230	910	730	770
	C.K.	730	1050	470	940	1040	220	240	1830	1390	780	280	510	420	450	610	1340	970	1090
	Mean	1530	1460	1050	970	1160	740	1160	1580	1320	1080	880	790	1120	1170	1140	1370	850	910
Urine ketones (mg/4 hr)	S.W.	0.0	0.0	2.8	2.3	19.7	31.6	20.6	11.4	15.8	18.5	50.0	69.6	53.6	18.6	30.0	15.1	26.1	60.4
	W.D.	0.9	0.0	3.8	3.7	9.1	20.4	4.3	2.9	8.9	11.2	53.2	19.9	46.0	10.2	29.2	37.2	95.6	84.8
	S.J.	0.0	0.0	0.6	2.5	2.0	3.4	7.4	4.0	4.8	5.5	6.9	12.3	14.5	8.9	9.8	7.3	31.9	20.8
	R.A.	0.0	0.0	1.4	0.6	4.4	7.4	3.7	2.2	2.3	6.1	12.1	23.6	10.7	14.1	11.9	29.4	23.4	86.8
	C.K.	0.0	0.0	1.4	5.5	7.2	1.0	0.7	2.2	10.9	3.9	7.3	9.2	1.9	5.4	17.8	15.4	26.5	33.4
	Mean	0.2	0.0	2.0	2.9	8.5	12.8	7.3	4.5	8.5	9.0	26.0	27.0	25.3	11.4	19.7	20.9	40.7	57.2

TABLE 4

Statistical significance by analysis of variance of the day to changes of the blood and urine levels in the healthy and diabe the three-day starvation period. Only the probability values which are significant at the 5 percent level are shown.

MOIETY	GROUP	DAY TO DAY VARIATION Day 1 vs Days 2 + 3 (p)	Day 2 vs Day 3 (p)
Blood Glucose	Healthy	<0.001	--
	Diabetic	<0.001	<0.01
Plasma Cortisol	Healthy	<0.001	--
	Diabetic	--	<0.05
Serum Insulin	Healthy	--	--
	Diabetic*	--	--
Serum Phosphate	Healthy	<0.01	--
	Diabetic	<0.001	--
Urine Phosphate	Healthy	<0.05	--
	Diabetic	<0.001	--
Urine Urea Nitrogen	Healthy	--	--
	Diabetic	--	--
Serum Free Fatty Acids	Healthy	<0.001	<0.01
	Diabetic	<0.001	--
Serum Ketones	Healthy	<0.001	<0.001
	Diabetic	<0.001	<0.001
Urine Ketones	Healthy	<0.001	<0.01
	Diabetic	<0.001	<0.001
Serum Triglycerides	Healthy	--	<0.05
	Diabetic	<0.001	<0.001

* 3 subjects (see text)

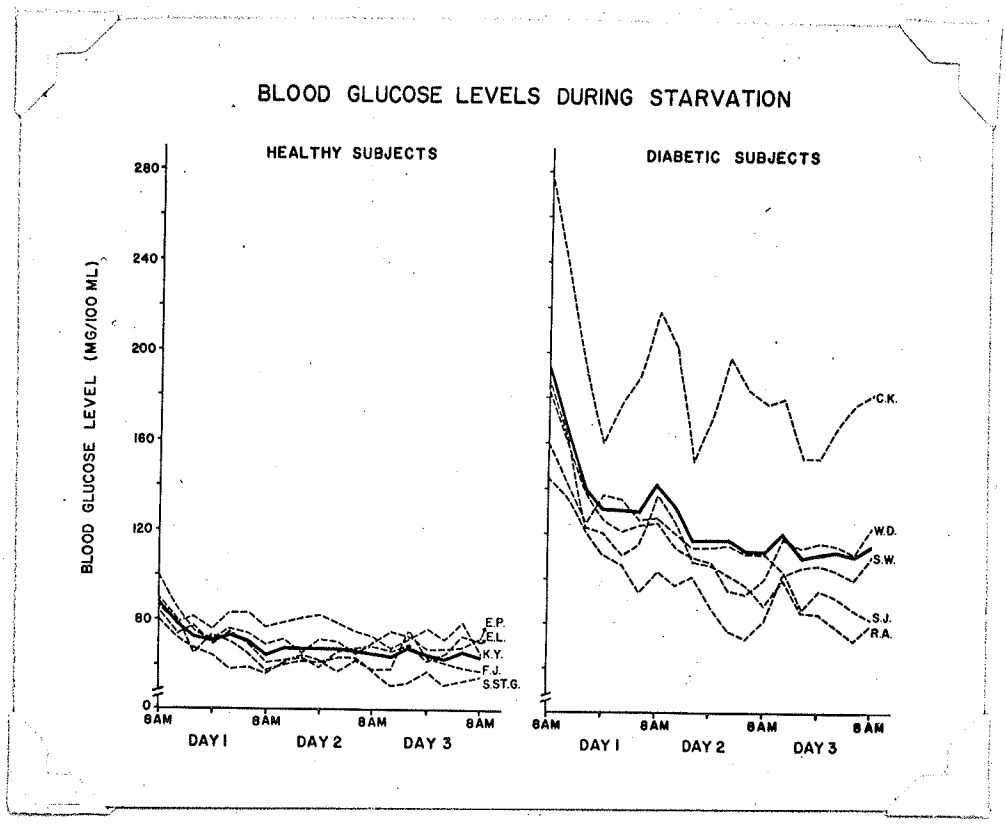

FIGURE 1 Individual (---) and mean (—) blood glucose levels in the healthy and diabetic subjects during the three-day starvation period.

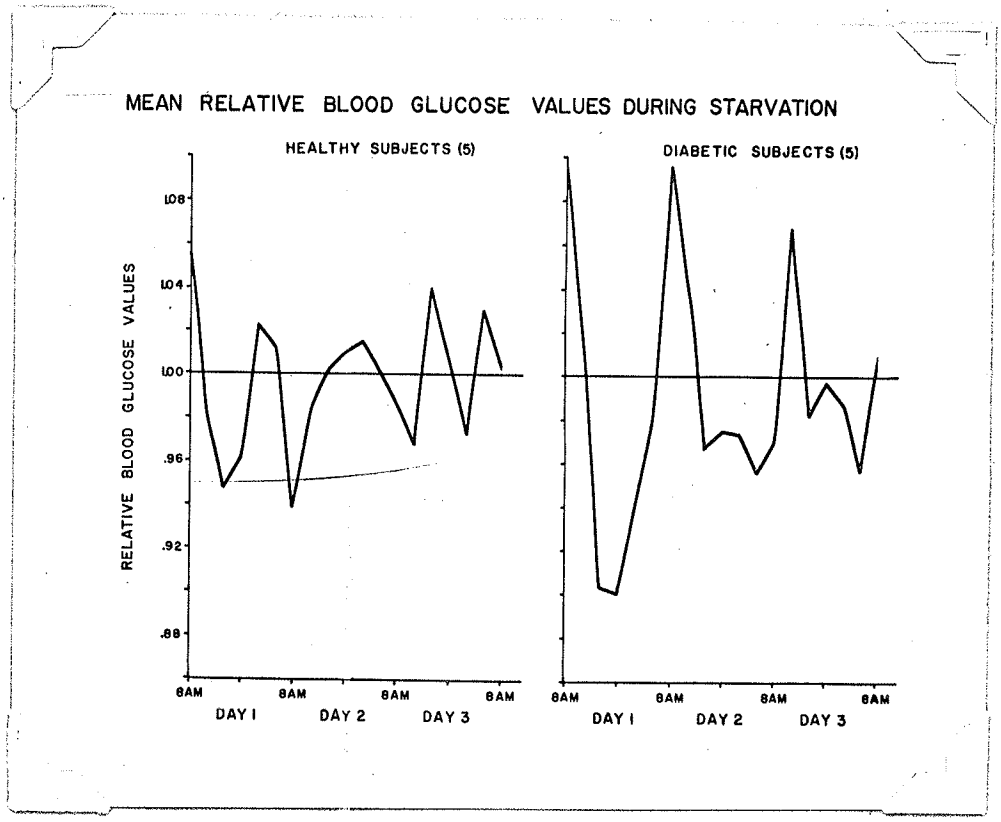

FIGURE 2 Mean relative blood glucose values in the healthy and diabetic subjects during the three-day starvation period.

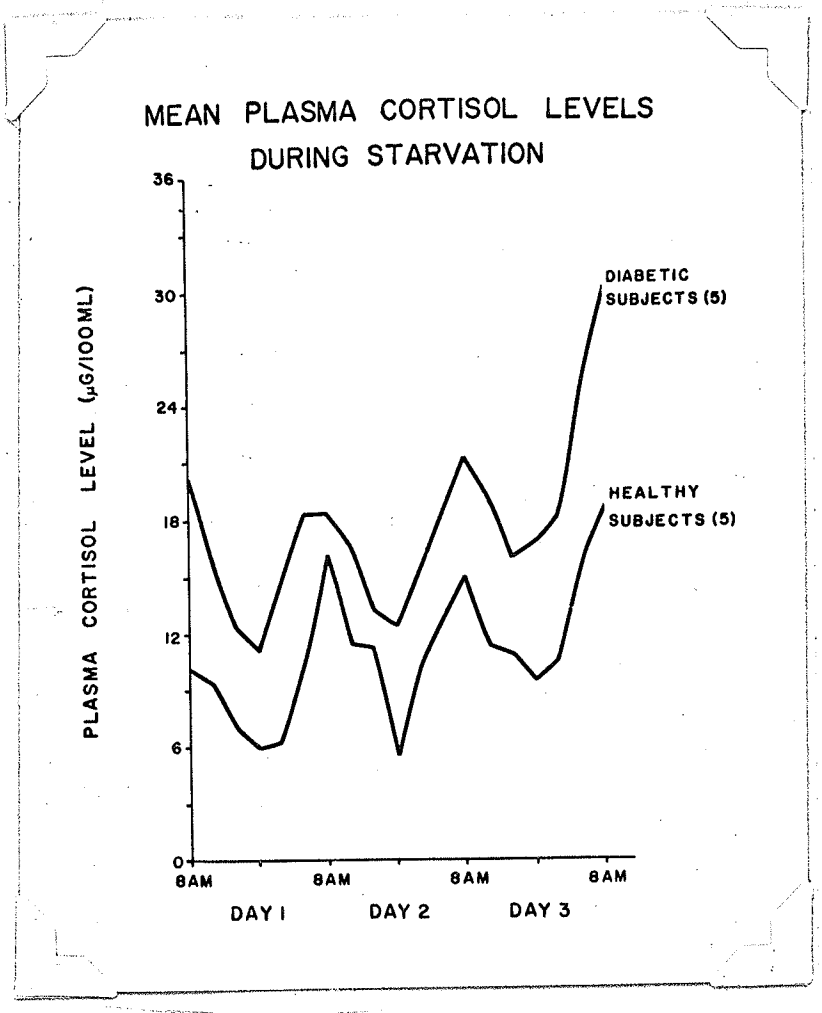

FIGURE 3 Mean plasma cortisol levels in the healthy and diabetic subjects during the three-day starvation period.

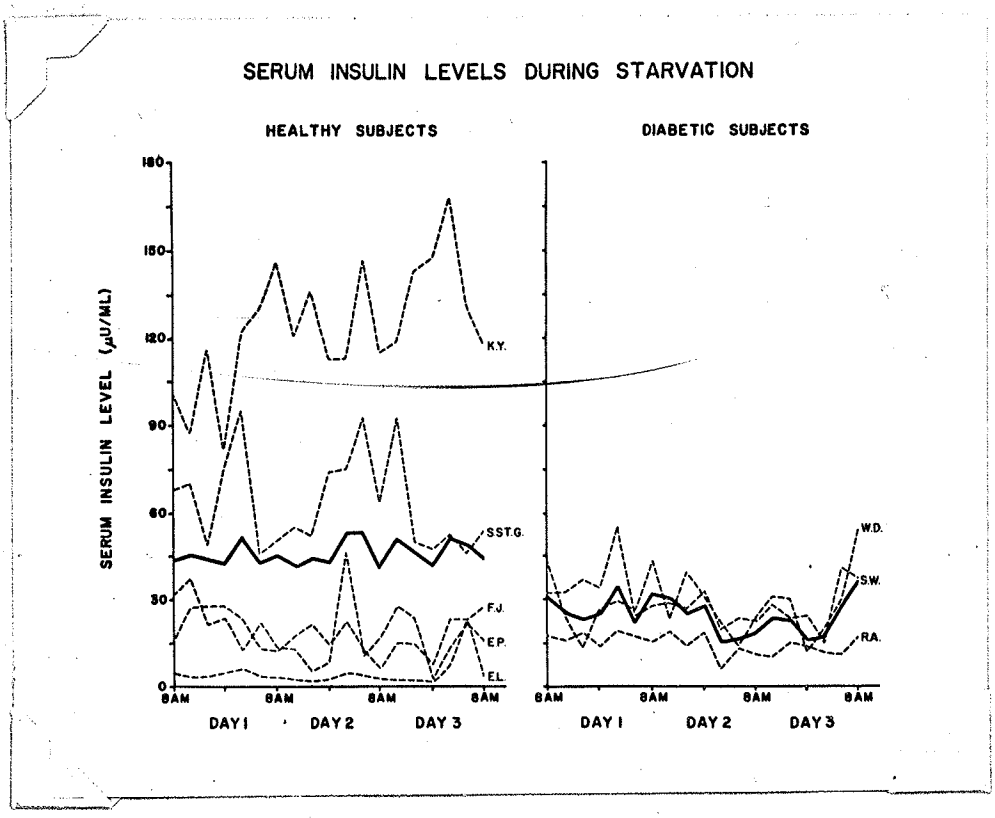

FIGURE 4 Individual (---) and mean (___) serum insulin levels in the healthy and in three of the diabetic subjects during the three-day starvation period.

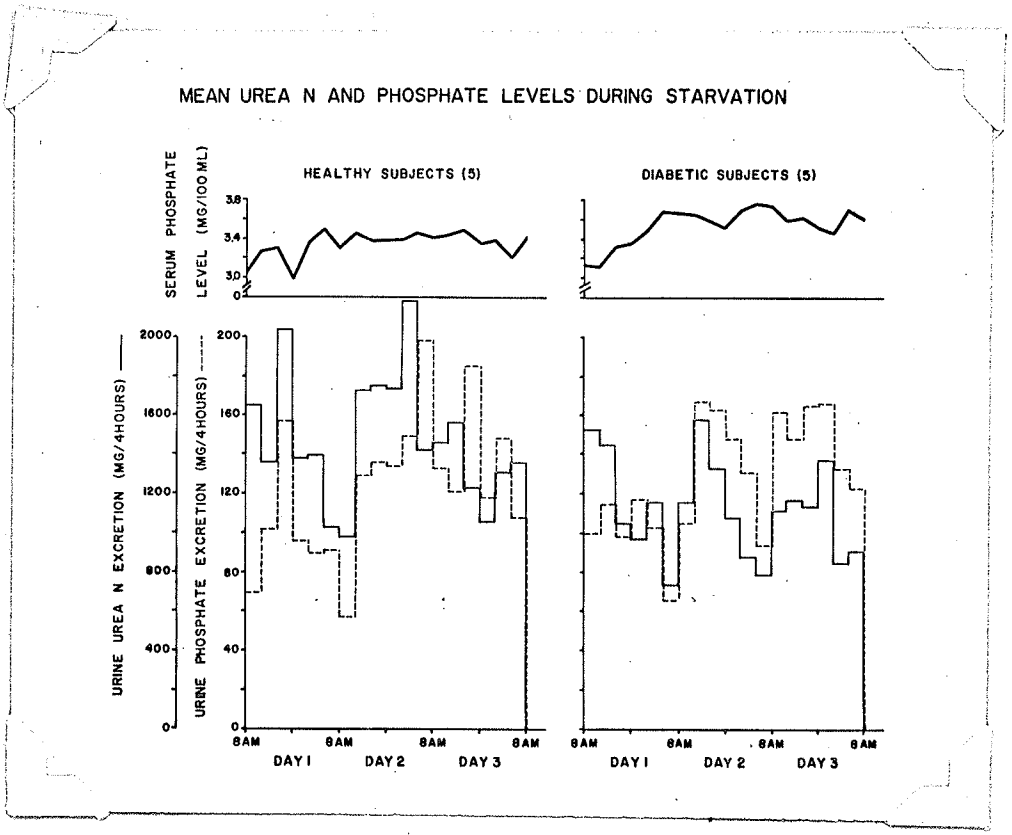

FIGURE 5 Mean serum and urine inorganic phosphate levels, and urine urea nitrogen levels, in the healthy and diabetic subjects during the three-day starvation period.

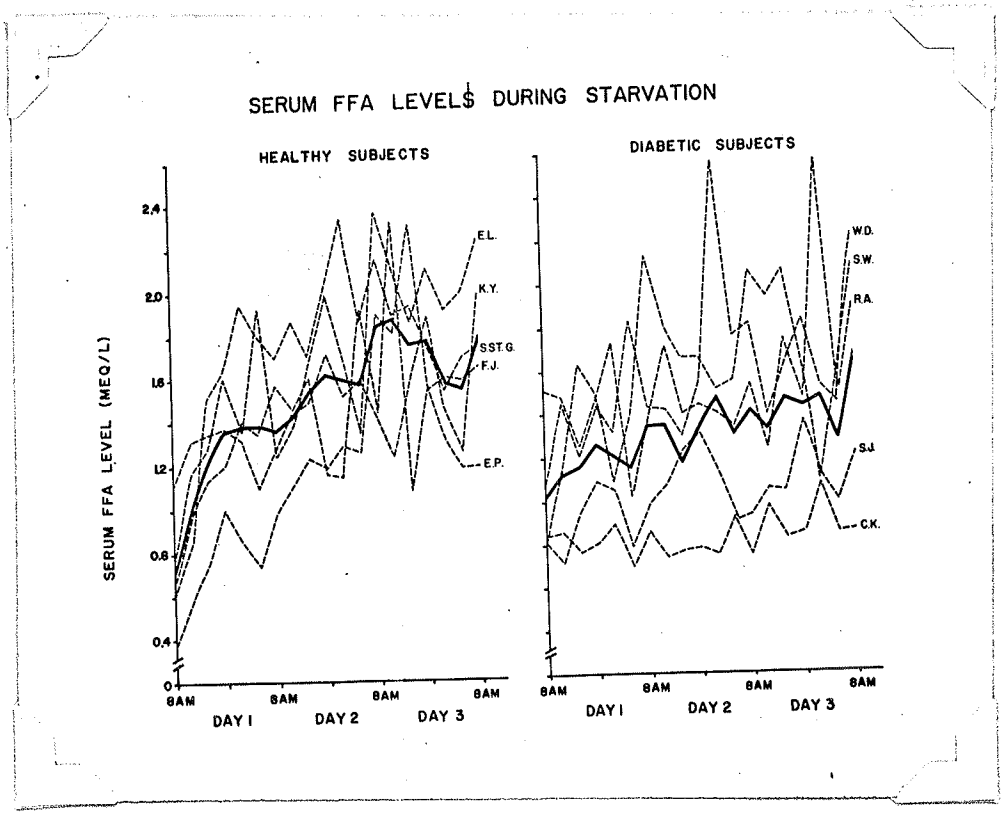

FIGURE 6 Individual (---) and mean (——) serum free fatty acid levels in the healthy and diabetic subjects during the three-day starvation period.

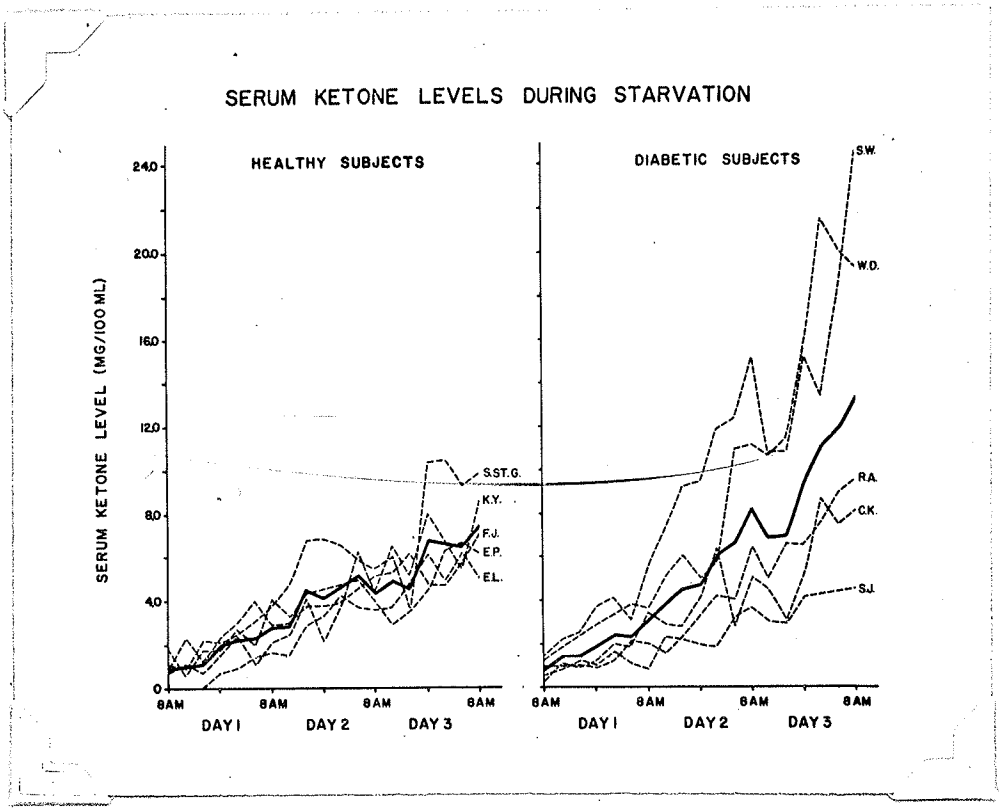

FIGURE 7 Individual (---) and mean (——) serum ketone levels in the healthy and diabetic subjects during the three-day starvation period.

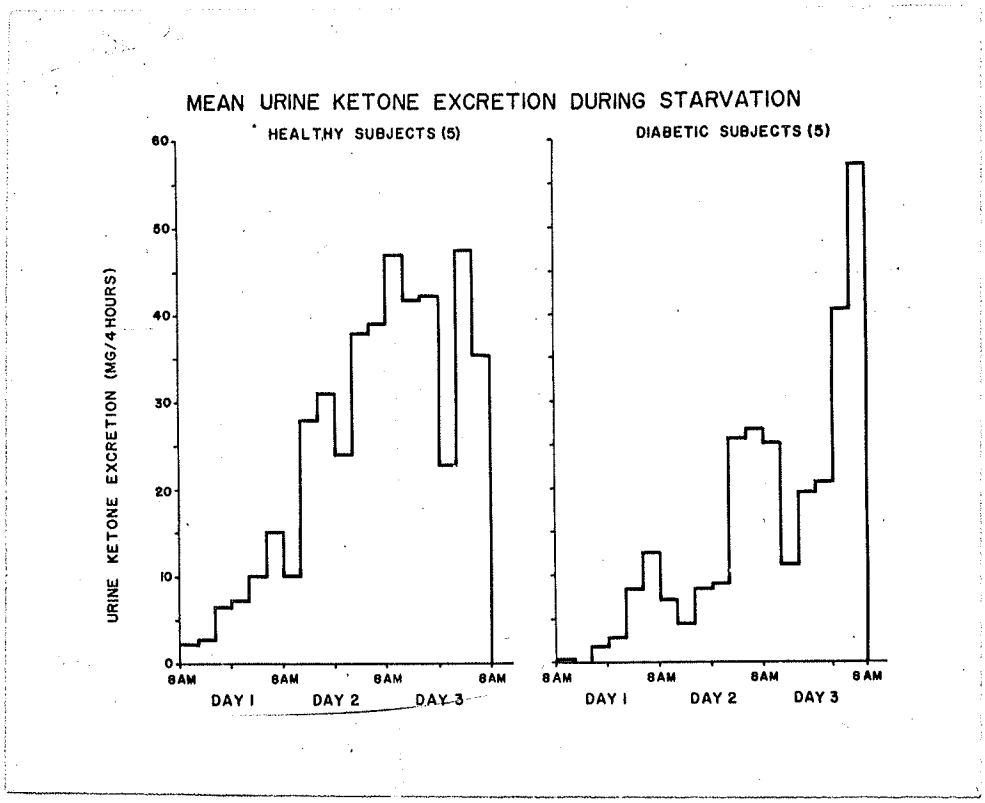

FIGURE 8 Mean urine ketone levels in the healthy and diabetic subjects during the three-day starvation period.

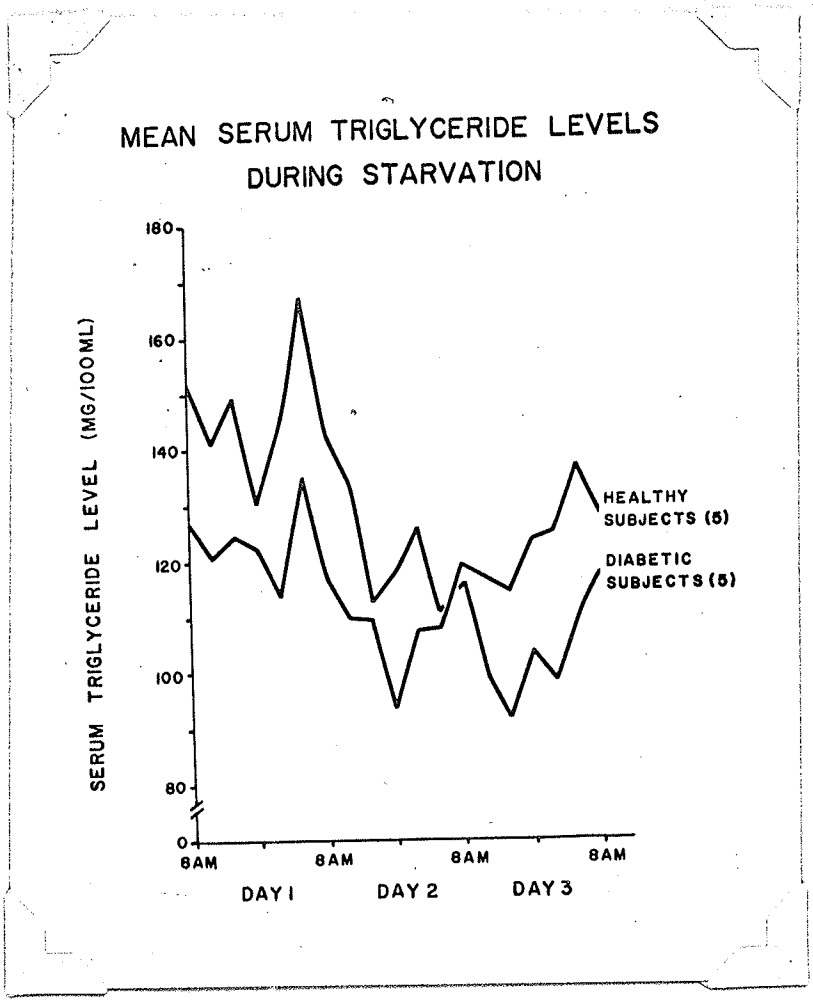

FIGURE 9 Mean serum triglyceride levels in the healthy and diabetic subjects during the three-day starvation period.