SOME ASPECTS OF INTERMEDIARY METABOLISM

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

PART II. THE HORMONAL REGULATION OF ADIPOSE TISSUE METABOLISM: A REVIEW

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INTRODUCTION

Whereas in healthy subjects the blood glucose level remains practically constant during short periods of fasting, in untreated diabetic subjects it usually declines. 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 commonly recognized, this matter seemed worthy of further study.

An overnight rise in blood glucose levels during fasting

[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.

in diabetic subjects was first observed by Hatlehol in 1924 (2). Later Möllerström 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 maximum levels in the morning. In spite of these various studies, and the recent interest in the general field of diurnal biorhythms (8-13), the endogenous periodicity of carbohydrate metabolism is still not discussed in standard manuals of diabetic management.

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. The healthy subjects were three men and two women ranging in age from 51 to 63 years. One of these subjects was overweight. The diabetic subjects were two men and three women ranging in age from 47 to 71 years. Three of these subjects were overweight. 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), and the results expressed as pork insulin equivalents. 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), declined from 0.86 $\pm$ S.D. 0.05 on the first day to 0.71 $\pm$ S.D. 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 subjects 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 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

[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.

and level ($r = 0.85$, $p < 0.01$) has previously 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.

No glucose was present in the urine of the healthy subjects. Only two of the diabetic subjects had glycosuria, which was minimal and intermittent. The values were not suitable for statistical analysis.

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 μg/100 ml. In the diabetic

subjects, the mean initial level was 20 μg/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 μg/100 ml in the healthy subjects and 30 μg/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 widely 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 than 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 showed no 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 ($t = 2.55$, $p < 0.05$), 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 Möllerström (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 is probably 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 glyco-

gen cycle which has been observed in several species (25, 29). This glycogen cycle persists during starvation, and is altered by light-dark reversal (25). 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 is 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 hepatic 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. While a cycle in the serum free fatty acid levels would have been expected to accompany changing peripheral glucose utilization, its absence could 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 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 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 is consistent with the regulation of hepatic glycogen by plasma cortisol under the cyclic influence of adrenocorticotropic hormone (34, 41, 42). Evidence from studies of the liver glycogen cycle in adrenalectomized animals has been conflicting (8, 25). In order to further 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).

It seems 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 is probable that an ineffectual insulin response allowed the cycles to become manifest. The failure to demonstrate cyclic insulin levels in the peripheral venous blood may have been due to

trapping of endogenous portal venous insulin by the liver (52, 53).

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 withdrawal, 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 preceding 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 may play an adaptive role in starvation by enhancing gluconeogenesis. The finding that the serum cortisol level is also elevated in diabetes brings to mind the metabolic similarities between diabetes and starvation.

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.

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, probably 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. The increased cortisol level during starvation probably plays an adaptive role by enhancing gluconeogenesis. The finding of an elevated serum cortisol level in the diabetic subjects is consistent with the metabolic similarities between diabetes and starvation.

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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	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.
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 ketones (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 triglycerides (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

		Day 1						Day 2						Day 3					
Moiety	Sub-ject	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 phos-	F.J.	81	143	255	181	124	80	44	167	115	185	386	134	166	143	136	7	113	101
phate	S.S.G.	86	110	110	67	66	132	54	127	184	127	67	314	177	98	114	305	87	105
(mg/	E.P.	65	74	112	114	37	56	33	166	145	117	59	69	102	95	315	78	102	54
4 hr)	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	F.J.	1090	1950	4250	2860	2200	1210	750	2370	2500	3200	4890	1680	1590	1320	1610	340	1630	1804
urea	S.S.G.	1780	1370	1300	570	1040	880	1360	1420	1070	930	2170	1130	1050	840	420	1220	1170	1094
nitro-	E.P.	2320	1490	1900	1590	1580	1010	700	2070	2310	2030	960	1790	2210	2210	2500	1430	1580	1164
gen	E.L.	1680	1230	1420	650	1040	1140	1120	1660	1720	1360	1680	1390	1530	1900	660	1330	820	1558
(mg/	K.Y.	1360	730	1340	1240	1100	930	960	1140	1140	1170	1200	1110	910	1510	960	1000	1330	1170
4 hr)	Mean	1650	1350	2040	1380	1390	1030	980	1730	1750	1740	2180	1420	1460	1560	1230	1060	1310	1360
Urine	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
Ke-	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
tones	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
(mg/	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
4 hr)	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	18	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	28	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 free fatty acids (mEq/l)	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
	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
	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
	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
	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 ke-tones (mg/ 100 ml)	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.
	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.
	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
	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
	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.
Serum trygly-cerides (mg/ 100 ml)	S.W.	83	84	99	91	109	132	60	63	64	71	75	60	75	44	46	57	61	61	65
	W.D.	141	120	110	96	95	124	123	108	72	72	68	82	77	70	66	71	77	72	87
	S.J.	251	229	233	212	214	274	223	207	192	211	198	176	171	169	119	152	163	177	186
	R.A.	122	109	137	111	128	116	122	130	94	96	147	100	115	102	95	108	70	112	110
	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	Sub-ject	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 phos-	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
phate	S.J.	77	180	43	74	111	79	100	133	135	121	115	102	142	160	144	149	94	134
(mg/	R.A.	123	127	137	107	118	24	107	237	281	275	247	58	272	121	294	91	198	150
4 hr)	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	S.W.	1170	700	370	210	890	1010	1630	1470	1400	780	910	1020	1210	860	620	2280	930	760
urea	W.D.	1400	1080	1690	1100	1020	680	1460	1620	1010	1020	960	880	1320	1790	2260	1290	980	890
nitro-	S.J.	2530	2930	1300	1220	1940	900	980	1300	1280	1770	1320	800	1000	1170	1000	1030	660	1030
gen	R.A.	1840	1520	1430	1380	920	870	1480	1680	1540	1040	930	750	1630	1570	1230	910	730	770
(mg/	C.K.	730	1050	470	940	1040	220	240	1830	1390	780	280	510	420	450	610	1340	970	1090
4 hr)	Mean	1530	1460	1050	970	1160	740	1160	1580	1320	1080	880	790	1120	1170	1140	1370	850	910
Urine	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
ke-	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
tones	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
(mg/	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
4 hr)	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 da changes of the blood and urine levels in the healthy and (the three-day starvation period. Only the probability va which are significant at the 5 percent level are shown.

ıOIETY	GROUP	DAY TO DAY VARIATION Day 1 vs Days 2 + 3 (p)	Day 2 vs Day (p)	( )
ılood Glucose	Healthy	<0.001	--	
	Diabetic	<0.001	<0.01	
'lasma Cortisol	Healthy	<0.001	--	--
	Diabetic	--	<0.05	--
erum Insulin	Healthy	--	--	--
	Diabetic*	--	--	--
erum Phosphate	Healthy	<0.01	--	--
	Diabetic	<0.001	--	
'rine Phosphate	Healthy	<0.05	--	--
	Diabetic	<0.001	--	--
'rine Urea Nitro- gen	Healthy	--	--	--
	Diabetic	--	--	
erum Free Fatty Acids	Healthy	<0.001	<0.01	
	Diabetic	<0.001	--	--
erum Ketones	Healthy	<0.001	<0.001	
	Diabetic	<0.001	<0.001	
'rine Ketones	Healthy	<0.001	<0.01	--
	Diabetic	<0.001	<0.001	
erum Triglycer- ides	Healthy	--	<0.05	--
	Diabetic	<0.001	<0.001	--

* 3 subjects (see text)

TABLE 5

mmary of the day to day and within day changes of the blood and urine levels the healthy and diabetic subjects during the three-day starvation period.

IETY	GROUP	VARIATION IN DAY TO DAY LEVELS*	DIURNAL CYCLE PEAK TIME
od Glucose	Healthy	↓	--**
	Diabetic	↓	8 a.m.
sma Cortisol	Healthy	↑	8 a.m.
	Diabetic	↑	8 a.m.
um Insulin	Healthy	↔	--
	Diabetic	↔	--
um Phosphate	Healthy	↑	--
	Diabetic	↑	4 a.m.
ne Phosphate	Healthy	↑	--
	Diabetic	↑	12 n. - 12 mn.
ne Urea Nitrogen	Healthy	↔	--
	Diabetic	↔	8 am. - 8 pm.
um Free Fatty Acids	Healthy	↑	--
	Diabetic	↑	--
um Ketones	Healthy	↑	--
	Diabetic	↑	--
ne Ketones	Healthy	↑	--
	Diabetic	↑	4 am. - 8 am.
um Triglycer-ides	Healthy	↓↑	--
	Diabetic	↓	4 am. - 8 am.

* ↓ decline
↑ rise
↔ no change
↓↑ decline then rise

* -- no cycle present

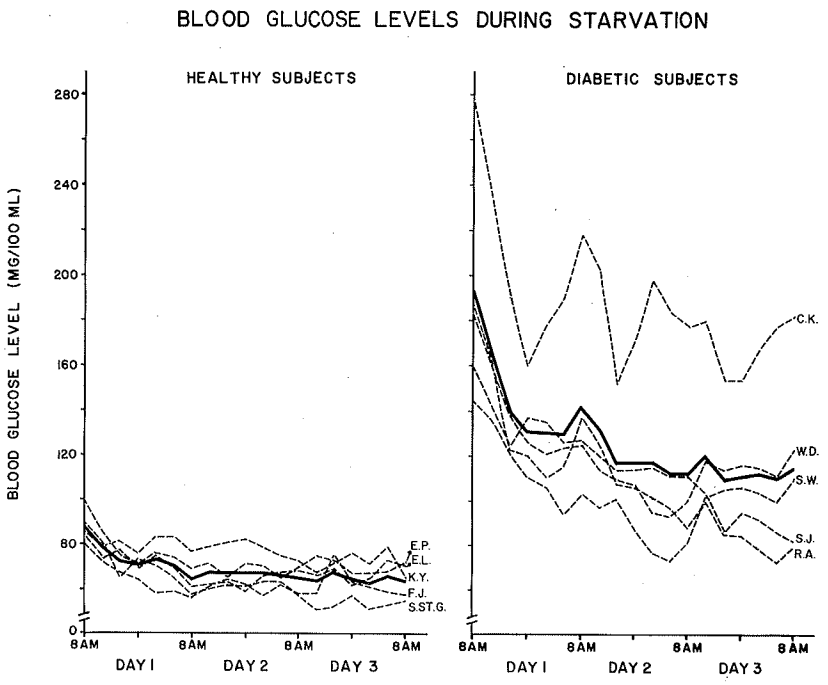

FIGURE 1 Individual (---) and mean (——) blood glucose levels in the healthy and diabetic subjects during the three-day starvation period.

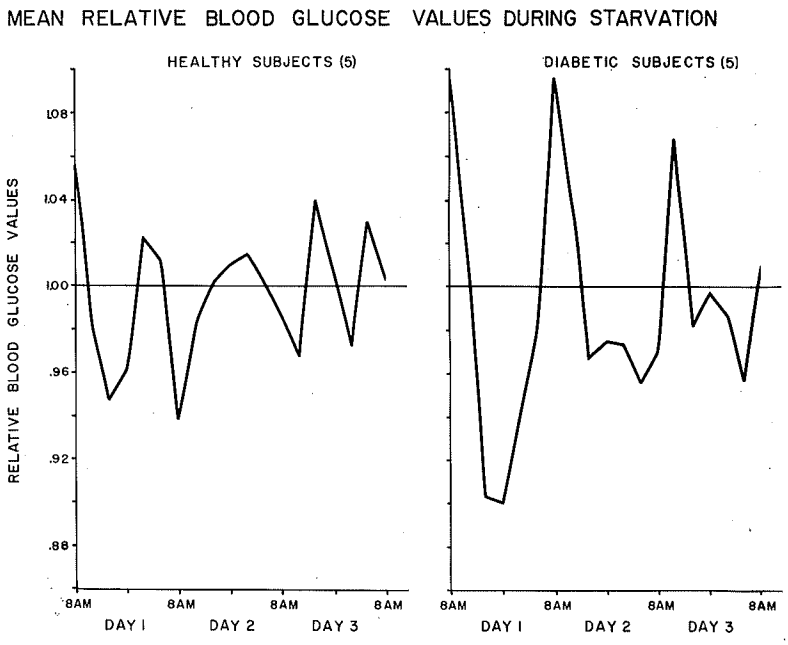

FIGURE 2 Mean relative blood glucose values in the healthy and diabetic subjects during the three-day starvation period.

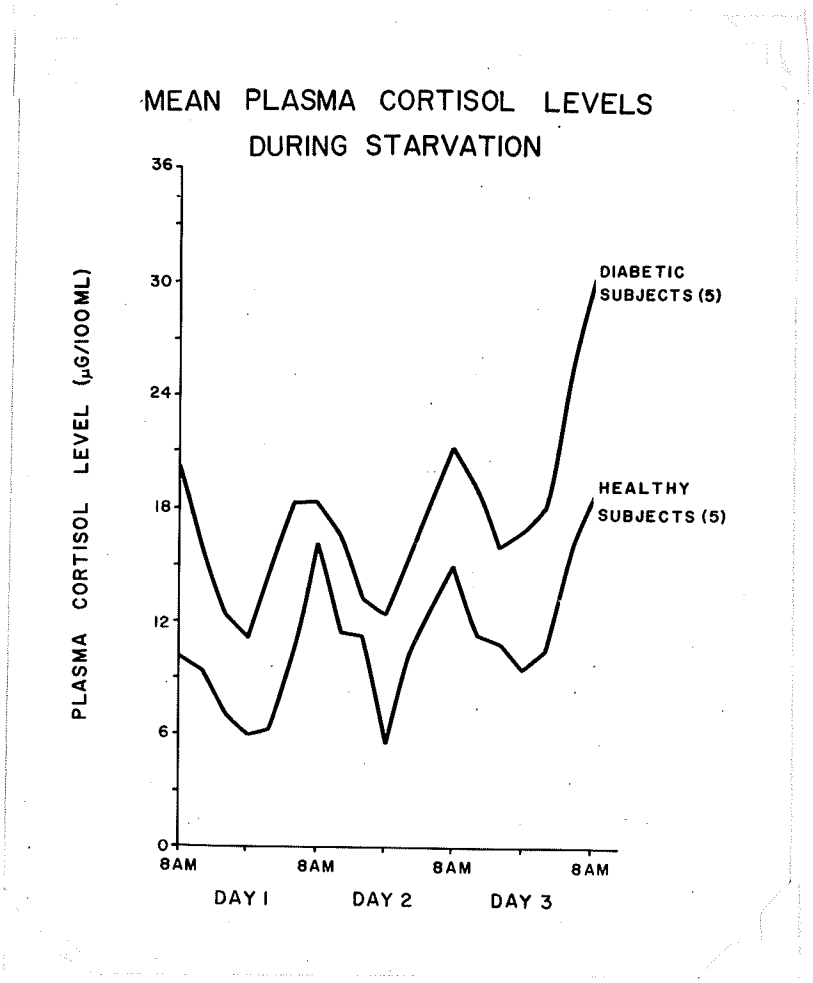

FIGURE 3 Mean plasma cortisol levels in the healthy and diabetic subjects during the three-day starvation period.

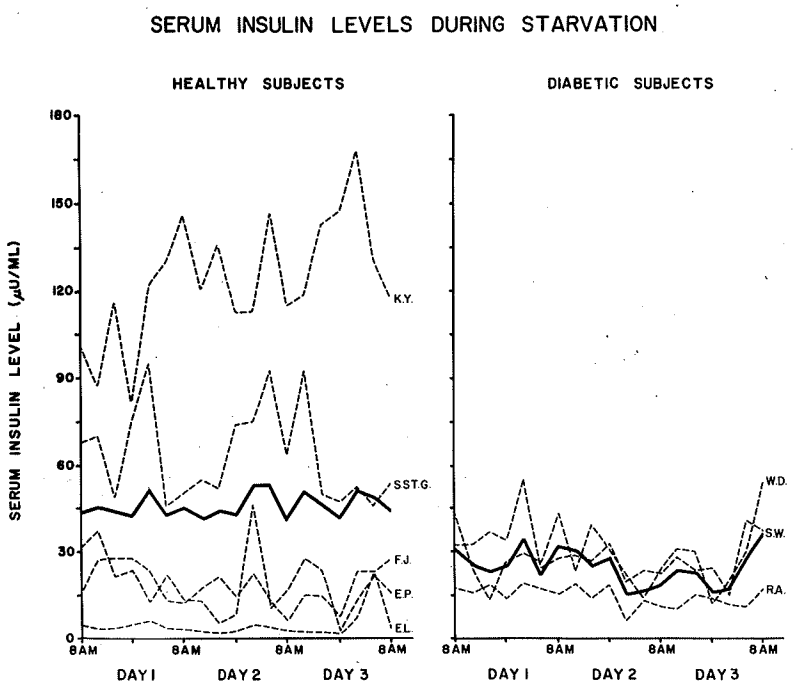

FIGURE 4 Individual (- - -) and mean (——) serum insulin levels in the healthy and in three of the diabetic subjects during the three-day starvation period.

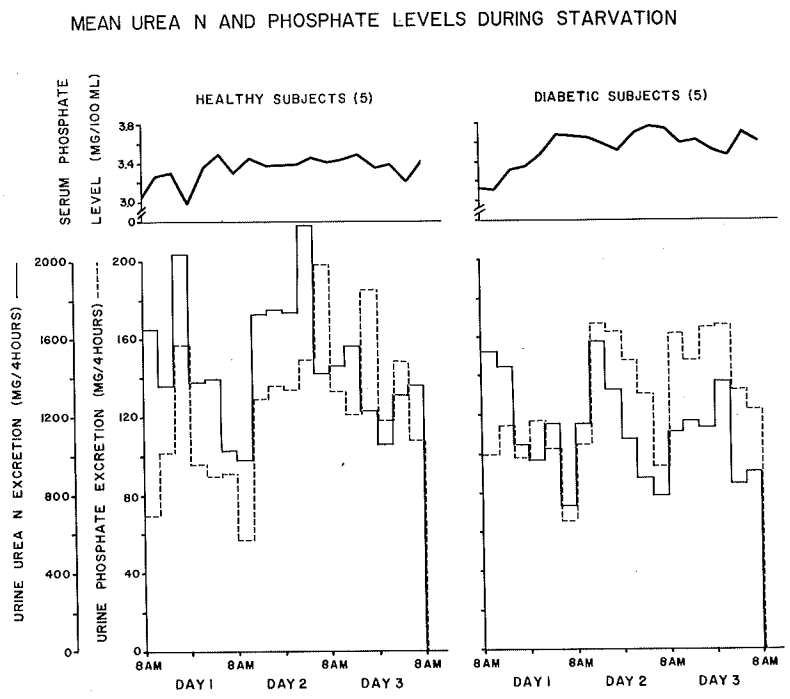

FIGURE 5 Mean serum and urine inorganic phosphate levels, and urine urea nitrogen levels, in the healthy and diabetic subjects during the three-day starvation period.

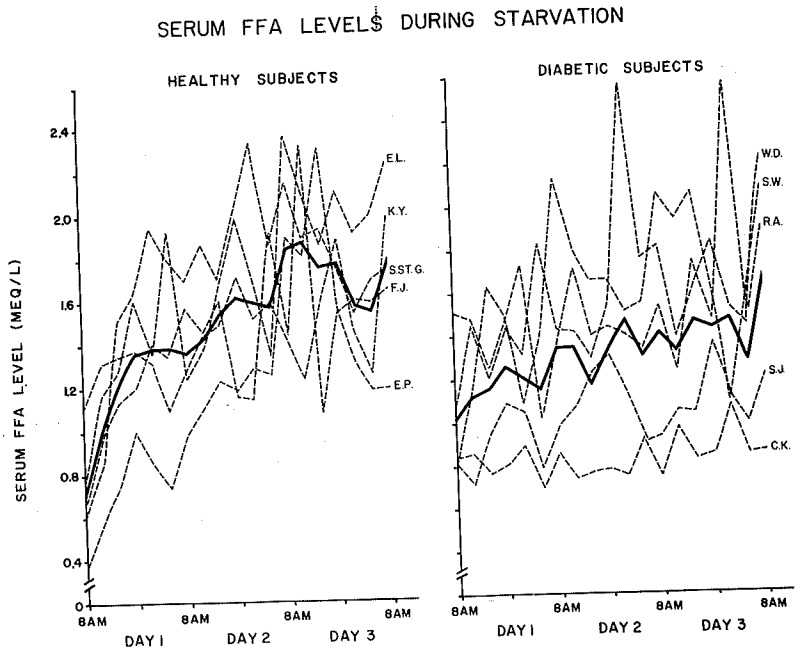

FIGURE 6 Individual (---) and mean (——) serum free fatty acid levels in the healthy and diabetic subjects during the three-day starvation period.

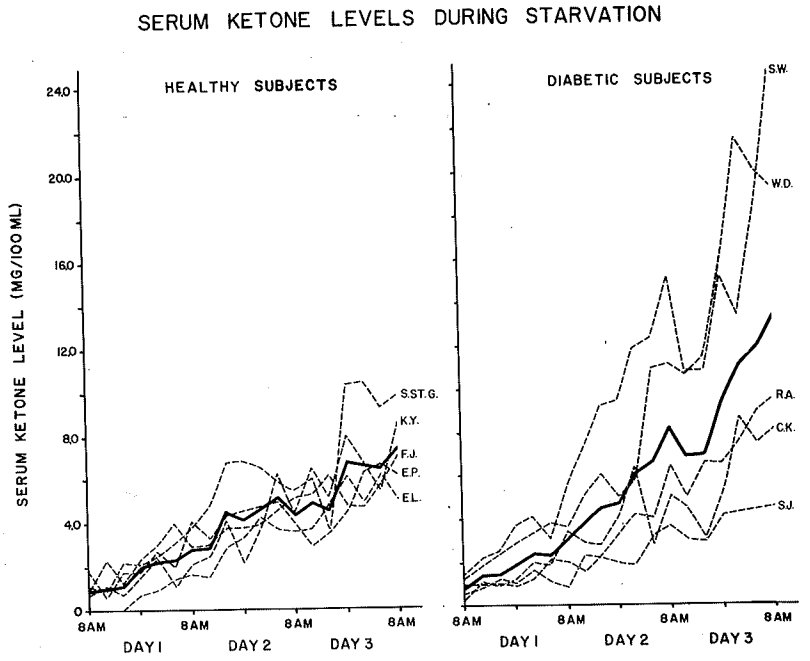

FIGURE 7 Individual (---) and mean (——) serum ketone levels in the healthy and diabetic subjects during the three-day starvation period.

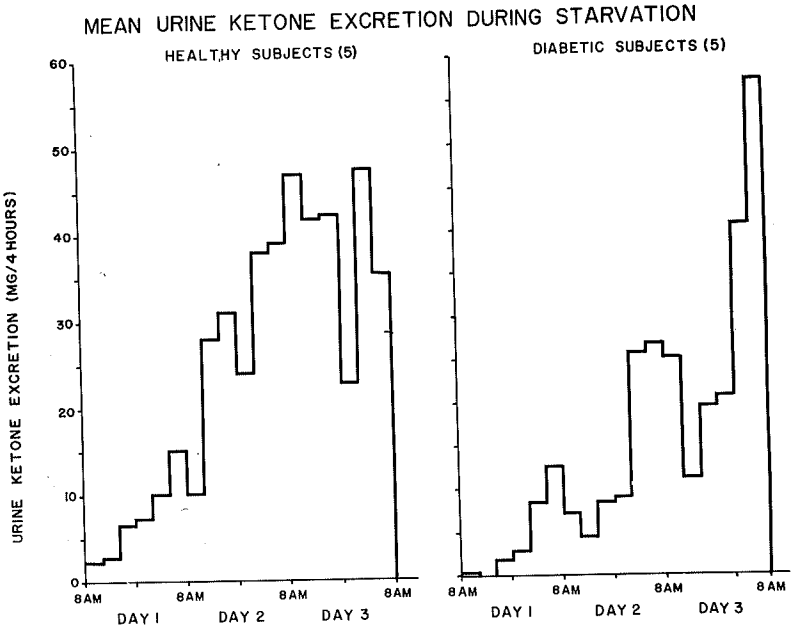

FIGURE 8 Mean urine ketone levels in the healthy and diabetic subjects during the three-day starvation period.

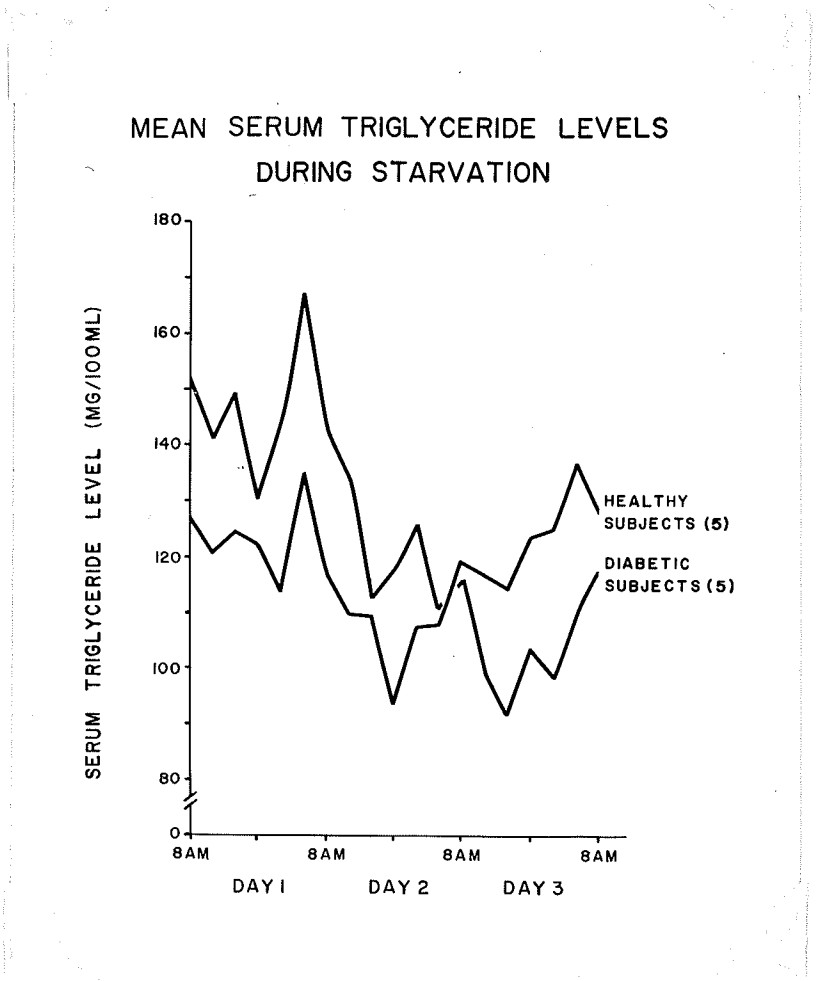

FIGURE 9 Mean serum triglyceride levels in the healthy and diabetic subjects during the three-day starvation period.

PART II. THE HORMONAL CONTROL OF ADIPOSE TISSUE METABOLISM : A REVIEW

PART II. THE HORMONAL CONTROL OF ADIPOSE TISSUE METABOLISM

1. INTRODUCTION

The physiological role of adipose tissue in the provision and storage of energy for the body has been poorly elucidated until quite recently. Less than 35 years ago, adipose tissue was regarded as metabolically inert, its function limited to insulation and support, and the passive storage of fat. Even after the classical studies of Schoenheimer and Rittenberg in 1936 (1) had shown that depot fat was in a true dynamic state, its role in energy metabolism continued to be accounted of far less importance than that of the liver. More recently, the era of enlightenment regarding the metabolic role of adipose tissue was furthered by the review of Wertheimer and Shapiro in 1948 (2). They concluded that in adipose tissue deposition and mobilization of fat were active processes involving the metabolism of the tissue, that synthesis of new fatty acids from carbohydrate proceeded continuously, and that these metabolic activities were regulated by nervous and endocrine factors. In 1956, Dole (3) and Gordon and Cherkes (4) independently

discovered that the free fatty acids (FFA)[1] constitute a major transport form for energy metabolism. In the intervening years, with the tools for measuring this elusive plasma fraction now available, the progress in our understanding of adipose tissue metabolism has been extremely rapid.

In the general review which follows no attempt will be made to cite all the original publications on which it is based. The reader is referred also to the many reviews on fat metabolism in the literature in the last five years (5-17).

[1] The term free fatty acids (FFA) refers to long-chain fatty acids not covalently bonded in the serum which are largely complexed with albumin. Other synonymous designations in the literature are non-esterified fatty acids (NEFA) and unesterified fatty acids (UFA).

2. THE METABOLISM OF ADIPOSE TISSUE

A) GLUCOSE METABOLISM

Glucose utilization in adipose tissue as in muscle is regulated by the supply of intracellular glucose -6- phosphate. This, in turn, depends on the transport of glucose across the cell membrane and its phosphorylation in the presence of glucokinase and ATP. Phosphorylation of glucose is unlikely to be the rate-limiting step because adipose tissue glucokinase is saturated at extremely low concentrations of glucose. It is therefore probable that the control of glucose metabolism is exerted at the level of glucose transport across the cell membrane. However, the phosphofructokinase step resulting in the conversion of fructose -6- phosphate to fructose-1, 6-diphosphate in the Embden-Meyerhof (glycolytic) pathway has received attention as a focal point in the regulation of carbohydrate metabolism in the cell (18-20) (see Figure 1). The problem of transport is further complicated in adipose tissue by the existence of an active pinocytosis, whose metabolic function is uncertain (21).

Glucose -6- phosphate is metabolised mainly by glycolysis and via the pentose phosphate shunt. The shunt is particu-

larly active in adipose tissue and until recently, was believed to be the major source of the reduced coenzymes necessary for the synthesis of fatty acids from glucose. Flatt and Ball (22) have suggested that only about half the coenzymes required for lipogenesis are produced in the pentose cycle, the remainder being furnished during the conversion of triose phosphate to acetyl CoA. Some evidence has accumulated to suggest that an uronic acid pathway may also be operative to a minor extent in adipose tissue (23). Glycogen storage and mobilization are of relatively little importance in this tissue. Most of the carbon from labelled glucose is found in carbon dioxide, glyceride-glycerol and fatty acids. Alpha-glycerophosphate, the precursor of glyceride-glycerol, is formed by reduction of dihydroxyacetone phosphate in the presence of reduced NAD(DPN). Because of the absence of glycerol kinase in adipose tissue, glycerol made available by triglyceride breakdown cannot be further utilized, and the rate of esterification of FFA is thus regulated by the level of glucose metabolism in the tissue. The glycerol is returned to the liver where it may be synthesized back into glucose and glycogen or used for re-esterification of FFA.

B) FFA METABOLISM

The dynamic state of the plasma FFA as a major component of the fuel mixture for energy metabolism in a great many tissues has already been mentioned. The plasma FFA content is derived almost solely from adipose tissue, although small and insignificant amounts may arise from chylomicrons after a fat-containing meal. Most tissues are able to utilize FFA as a source of energy and their transport and turnover rate in blood is extremely rapid. Various investigators have found that the half-life in plasma of albumin-bound FFA injected intravenously into various species is about two to four minutes.

The mode of release of FFA from fat cells depends upon intracellular hydrolysis of triglyceride under the influence of a tissue (hormone-sensitive) lipase which should not be confused with the lipoprotein lipase found in very small concentrations in circulating blood and which is activated by heparin. Vaughan et al (24) have recently described three lipases in rat adipose tissue homogenates and have characterized them further. These are: 1. lipoprotein lipase; 2. hormone-sensitive lipase; and 3. monoglyceride lipase. The FFA released during lipolysis are bound immediately to plasma albumin for transport. Glycerol is

carried to the liver where it is utilized. Under normal conditions, triglycerides are believed not to enter the circulation from adipose tissue.

C) SYNTHESIS AND HYDROLYSIS OF TRIGLYCERIDES.

Adipose tissue triglycerides constitute the major storage form of oxidizable substrate in mammals. The body's reserves of carbohydrate, in the form of glucose and glycogen, are very limited and, except during intervals immediately following meals, the body subsists primarily on calories supplied by stored fat.

An outline of the synthesis and hydrolysis of adipose tissue triglyceride is presented in Figure 2. The sources of adipose tissue triglyceride are several. Triglycerides, carried in chylomicrons and also in low-density lipoproteins can be incorporated into the depot fat. Although there is evidence that triglycerides can initially be taken up intact, they are mainly broken down by lipoprotein lipase to glycerol and FFA presumably at the cell surface, with only the latter being retained in the depot triglyceride. Circulating FFA per se, does not appear to be a major input form. Glucose is probably the major precursor of adipose tissue triglyceride, giving rise to both the glycerol moiety, by way of alpha-glycerophosphate and to the

fatty acid moieties by way of acetyl CoA. This formation of fat from non-fat precursors is termed lipogenesis.

In contrast to the various input forms for triglyceride synthesis there is only one major output form, the FFA. These depend upon the hydrolysis of the stored triglycerides which is termed lipolysis. Many studied have confirmed the dynamic state of depot fat, that is triglyceride synthesis and breakdown go on continuously even in the steady state. If the rates of the triglyceride synthesis and breakdown are equal, then the fat stores will be maintained at a constant level. Net mobilization will occur if the equilibrium is upset by increasing the rate of breakdown or decreasing the rate of synthesis. On the other hand, net deposition will occur by decreasing the rate of breakdown or increasing the rate of synthesis.

Considerable amounts of glycerol are produced in adipose tissue by lipolysis of circulating as well as stored triglycerides. The rate of entry of glycerol into the blood probably depends on the rates of turnover of both adipose tissue triglycerides and plasma lipoproteins. The rate of glycerol release compared to FFA mobilization is further complicated (25). Firstly these moieties occupy different pool sizes and secondly, it appears

that the lipolysis in adipose tissue may be incomplete with retention of glycerol as monoglyceride or diglyceride and their subsequent reesterification. It has been estimated, in the rabbit, that about 10 percent of the calories used may be derived from glycerol and this may be considerably greater during starvation (26).

D) THE GLUCOSE-FATTY ACID CYCLE.

The interplay of biochemical and hormonal factors that effects the reciprocal control of the glucose and fatty acid interrelationship is of fundamental importance in the homeostasis of energy metabolism. This relationship has been further clarified by Randle et al (27) in the form of a glucose-fatty acid cycle (Figure 3) for which some persuasive evidence is provided. In adipose tissue, the entrance of glucose, enhanced by the presence of insulin, leads to the immobilization of intracellular fatty acids by providing the alpha-glycerophosphate for esterification to triglyceride. Under these conditions, the plasma FFA concentration falls and remains low as long as glucose is available. On the other hand failure of the glucose supply, or the presence of any other factors which enhance lipolysis, results in FFA release and a rise in plasma FFA

concentration. Because fatty acids perfuse freely into cells, including skeletal and cardiac muscle, this leads to a rising intracellular level. These circumstances restrict the metabolism of glucose in muscle tissue largely by inhibiting the transport of glucose through the mechanism of insulin insensitivity. Thus, a mechanism is present whereby glucose and FFA mutually inhibit each other's metabolism. Its most important function resides in the carbohydrate-sparing effect during periods such as fasting and exercise so as to retain the integrity of those tissues with an obligatory dependence upon glucose, chiefly the central nervous system and renal medulla.

Randle and his co-workers, on the basis of further evidence have extended this mechanism to postulate that diabetes mellitus may result secondarily from an inherently abnormal increase in FFA release in this disease (20, 27, 28). The finding of enhanced lipolysis in diabetes lends support to this hypothesis (29, 30). Some evidence has subsequently appeared in the literature which casts some doubt on this hypothesis (31) and similar evidence can also be gleaned from the data of Unger et al (32). The final answer, therefore, is not forthcoming at the present time, and it appears that because of the

"cyclic" nature of the interrelationships between carbohydrate and fat metabolism, there will be some difficulty in trying to assess which is the primary cause and which is the secondary effect.

3. HORMONAL AND NEURAL CONTROL OF FAT MOBILIZATION

Most of the hormonal effects in adipose tissue can be explained on the basis of two principal sites of control. They are first the transformation of extracellular glucose into intracellular glucose -6- phosphate and second the activity of the intracellular (hormone-sensitive) lipase system responsible for the breakdown of stored triglycerides.

A) INSULIN, GLUCOSE AND STARVATION.

The primary effect of insulin is thought to be at the level of transport of glucose across the cell membrane. Synthesis of glycogen, glycolysis, oxidation in the pentose cycle and synthesis of fatty acids are all increased. The production of increased amounts of alpha-glycerophosphate promotes storage of fatty acids as triglyceride and inhibits their release into the circulation. Similar effects are produced by increasing the concentration of glucose to which the tissue is exposed, provided some insulin is present.

While insulin is certainly the most important agent regulating glucose metabolism and lipogenesis it should be noted that similar effects have been produced under certain experimental conditions with prolactin and with oxytocin.

Thus during starvation, where an inadequate supply of glucose exists, and hence a diminished source of alpha-glycerophosphate is available, the equilibrium between lipolysis and reexterification is disturbed so that net lipolysis occurs. That the primary defect lies in diminished reesterification has been abundantly confirmed and it has been shown that the adrenal cortex, adrenal medulla, pituitary or thyroid are not required for the enhanced lipolysis observed during starvation (33, 34). However, the findings during starvation of elevated serum levels of glucagon (32), growth hormone (35), and cortisol (36, 37), and a urine fat mobilizing substance (17), require some further study. These may be related to the observed activation of adipose tissue lipase during starvation, the cause of which remains unexplained (17). Fatty acid synthesis itself is impaired as the result of a number of factors during starvation, including the diminished generation of reduced NADP (TPN) in the pentose cycle, the absence of an hepatic lipogenic stimulator, and the probable presence of an hepatic lipogenic inhibitor (38).

Similar to the metabolic effects of starvation, the "insulin lack" in diabetes mellitus results in inhibition of glucose uptake and lipogenesis, and favors the release of FFA into

the circulation. The possibility of a primary disturbance resulting in an enhanced tissue lipolytic activity in diabetes has already been mentioned. Recent work (39) suggests that insulin may also play a role in inhibiting the epinephrine-sensitive lipase of adipose tissue, and thus insulin deficiency would be expected to result in an enhanced lipolytic activity. It should be further noted that the finding of increased plasma corticosteroid levels in diabetic sera has led observers to suggest that this may be the cause of the altered lipid metabolism in diabetes (36, 37). Thus, the increased FFA levels observed in diabetes are a result of increased production. Data has accumulated to suggest an increased FFA utilization as well (6, 40), which, if anything, would tend to minimize this lipolytic effect.

B) LIPOLYTIC SUBSTANCES.

The following factors have been shown to stimulate the release of FFA in vitro, in vivo, or both (14, 16, 17, 41-44).

1. Catecholamines - epinephrine and norepinephrine
2. Growth hormone
3. Adrenocorticotrophic hormone
4. Thyroid-stimulating hormone
5. Follicle-stimulating hormone
6. Alpha and beta intermedin (MSH)

7. Peptides I and II (Astwood) = Fraction H (Rudman)

8. Lipotropin (Li)

9. Fat mobilizing substance (FMS-Chalmers)

10. Arginine vasopressin

11. Glucagon

12. Adrenal glucocorticoids

13. Thyroxine

14. Testosterone

These agents, with the possible exception of cortisol, and thyroxine, liberate fatty acids from adipose tissue by directly augmenting the lipolytic reaction. Indeed an in vitro enhancement of tissue lipase activity has been observed with a number of these substances, most important of which are the catecholamines. The delayed rise in serum FFA after parental injections of growth hormone suggests that its action is an indirect one. Similarly, in vitro, a direct stimulation of lipolysis in physiological doses has not been demonstrated. It should also be noted that whereas the other pituitary peptides produce an immediate response in certain species, and are active in vitro, the only purified adenohypophyseal peptide that produces an increase in the serum FFA level of the fasted monkey

or man is primate growth hormone. Furthermore, the peptides derived from pituitary extracts - peptides I and II and lipotropin, - may not be native but rather altered by the extraction procedure.

During fasting or restriction of carbohydrate intake the urine of man and certain other mammalian species contains a potent adipokinetic peptide of low molecular weight (16, 17). The pituitary or adjacent hypothalamus are necessary for its production. It differs from known pituitary adipokinetic substances but its relation to growth hormone requires further elucidation.

The physiological significance of glucagon in stimulating lipolysis is similarly unknown (17). Although in vitro effectiveness has been demonstrated, its effect in elevating serum FFA levels after parental injection in man is delayed in time, and is preceded by a diminution in the levels. Although the exact mechanism requires further study, it appears that the initial fall in levels is not related to liver glycogenolysis or to insulin contamination, but rather to a direct enhancement of peripheral glucose utilization.

Cortisol appears to act mainly in a permissive fashion in stimulating lipolysis. The glucocorticoid hormone in some

unknown way conditions the metabolic stance of the adipose tissue to permit maximum response to catecholamines, notwithstanding the observations that under certain conditions glucocorticoids appear to exert a direct role in the enhancement of lipolysis. A similar permissive action has been ascribed to thyroxine as well. Recent evidence suggests that its lipolytic effect may result from induced formation of additional tissue lipase (17), as well.

A possible mode of action common to catecholamine, peptide hormones and glucagon is through the stimulation of adenyl cyclase with subsequent accumulation of cyclic 3'5'-AMP, but not only does there appear to be a poor correlation between cyclic AMP formation and lipolytic effect (45), but exogenous cyclic AMP added in vitro appears to be without effect.

There are considerable species differences to the responsiveness of adipose tissue to lipolytic hormones. Variation in sensitivity among different species may be related to inactivating enzymes. The presence of such an enzyme system in the rat's fat cell appears to ablate responsiveness to the intermedins, vasopressin, and fraction H, and to shorten the duration of response to adrenocorticotropic hormone (17).

Besides their primary effect on the lipolytic mechanism,

the adipokinetic hormones produce characteristic changes in glucose metabolism believed to be mediated by a rise in the intracellular FFA concentration. These should not be confused with the metabolic consequences of FFA accumulation in cardiac and skeletal muscle as previously discussed. In adipose tissue the following changes occur: 1. an increase in glucose uptake and an increase in the incorporation of glucose carbon into glyceride-glycerol, 2. a relative decrease in the metabolism of glucose over the pentose pathway and 3. an increase in oxidation of glucose by the Krebs cycle. In addition, in the presence of insulin there is an even greater increase in glucose incorporation into glyceride-glycerol, and the insulin effects on glycogen and fatty acid synthesis are inhibited. A large increase in oxygen consumption accompany these changes and it is possible that heat generated in this fashion may at times contribute to the maintenance of body temperature.

C) NEURAL CONTROL

It has been recognized for some time that the direct innervation of adipose tissue plays an important role in facilitating fat mobilization. This effect is mediated by noradrenaline release at the postganglionic sympathetic nerve endings. The

evidence on which these conclusions are based include the demonstration of sympathetic innervation of fat cells, the presence of considerable amounts of noradrenaline and its de novo synthesis in adipose tissue, the in vivo and in vitro lipolytic effect of catecholamines, the liberation of FFA by the electrical stimulation of sympathetic nerves in vitro, and the blocking of the lipolytic response by adrenergic blocking agents (17). The role of the sympathetic nervous system in determining basal rates of FFA release is not known, but its effect in increasing FFA release during emotional or cold stress appears to be reasonably well documented.

D) METABOLIC CONSEQUENCES OF FFA MOBILIZATION (14, 17)

Most of the lipolytic effects of these hormones can be simulated by the infusion of FFA-albumin complexes in vivo or by the perfusion of isolated organs or addition to tissue slices and homogenates in vitro.

1. Deposition of fat in the liver: This occurs as a direct consequence of an increase in FFA mobilization. It is enhanced by the presence of an intact pituitary-adrenal axis and deranged liver function.

2. Elevation of serum lipoprotein levels: Elevated

plasma levels of FFA, and the consequent elevated rate of uptake of FFA by the liver, lead in a rather direct manner to an increase in the rate of production and secretion of lipoproteins into the plasma.

3. Increase in rate of FFA oxidation: It appears that plasma FFA concentration is a factor controlling the rate of FFA oxidation much like the glucose level is a factor in determining its own rate of utilization. However, the hormonal effects on uptake and utilization of plasma FFA must still be evaluated.

4. Production of ketone bodies: The end results of enhanced mobilization of FFA appears to be in the conversion to ketone bodies in the liver either directly or after prior incorporation into the liver triglycerides.

5. Calorigenic effect: The hypothesis that the increased oxygen consumption observed following the administration of some of these hormones results secondarily from an enhanced lipolysis is attractive, and may be mediated by the known effects of FFA on the uncoupling of oxidative phosphorylation (6). The clinical counterparts may be seen in the increased oxygen consumption observed in hyperthyroidism and functioning

pheochromocytoma both of which are associated with increased circulating levels of FFA (46). Recent studies cast some doubt on this hypothesis (47), however. The administration of nicotinic acid blocks the FFA release by infused catecholamines in normal and triiodothyronine-treated subjects. Similarly glucose administration reduces FFA levels in both these groups. Despite these effects on FFA levels, no change in oxygen consumption was observed. However, it is still possible that during times of temporary reduction in the rate of delivery of FFA from the depots a "buffering reservoir" of stored tissue lipids may be called upon to act as substrate and hence the calorigenic response would continue unabated.

6. Effect on thrombosis: Available data suggest that enhanced lipid mobilization causing increased concentration of FFA in plasma may shorten the clotting time by inducing changes in the platelets. This may be of importance in the formation of thrombi in the intact organism.

4. PHYSIOLOGICAL ROLE OF HORMONES

The metabolic role of insulin in controlling the rate of FFA release appears extremely important. Insulin lack, as previously discussed, leads to a net increase in adipose tissue FFA release with the secondary consequences of "fatty liver", hyperlipemia and ketoacidosis. Similarly, a primary glucose lack, seen in starvation leads in a similar fashion to almost identical results. The presence of a functioning insulin feedback mechanism via ketone bodies in starvation, however, as recently described (48, 49), may serve as a control to FFA release so that the severity of the metabolic upset is markedly ameliorated and a progressive ketoacidosis does not result.

Although there is still much uncertainty about the physiological role of lipolytic hormones, it is becoming clear that the activity of the sympathetic nervous system functions in determining the levels of both FFA and other lipid fractions in plasma. The permissive role of glucocorticoids and thyroid hormone on the tonic activity of the sympathetic nervous system has previously been discussed. This tonic activity appears to result in the elevated FFA levels seen in the adaption to extrauterine life, in the adaptation to upright posture, during exercise,

and during cold or psychological stress.

Physical exercise produces a prompt and sustained rise in FFA turnover, correlating with the immediate release of catecholamines and perpetuated perhaps by growth hormone which is also released during exercise. The biological importance of these observations depends upon the now well-documented fact that both skeletal and cardiac muscle can function normally using fatty acids or ketone bodies as substrate. However, the finding that FFA levels still rise during exercise in adrenalectomized animals maintained on corticosteroids, and in patients with panhypopituitarism, casts doubt on the role played by catecholamines and growth hormone in this regard. More recent work suggests that sympathetic innervation results in a resistance to muscular fatigue. It is speculated that this is accomplished by the activation of intracellular phosphorylase or lipase.

Cold exposure similarly results in elevated plasma FFA levels associated with catecholamine release. In elegant studies in rats, Maickel et al (17) have shown that this FFA response is mediated by norepinephrine release and that its activation of adipose tissue lipase is maximal and is not further

enhanced by endogenous epinephrine. It has also been demonstrated that the elevated adrenocorticotropic and corticosteroid levels are of no importance in the FFA mobilization during cold exposure. Thyroid stimulating hormone and thyroxine probably do exert some influence during more prolonged exposure to cold.

The extent to which pituitary hormones are more directly involved in fat mobilization is less clear and may well vary in different species. The phenomenon of growth in immature animals, under at least partial control of growth hormone, is accompanied by elevated FFA levels presumably related to growth hormone secretion, and related to the supply of energy for growth. This problem may further be elucidated. The combined action of insulin and growth hormone on increasing protein anabolism is probably important in the growth of animals. Their mutual antagonism on FFA storage and release may serve to provide the actively growing body with FFA as energy, instead of its increased production and storage in the fat depots as seen with insulin alone.

The recent finding by Unger et al (50) of a sex-difference in fasting growth hormone levels, with levels far greater in females than in males, also lends support to its physiological

role in man when viewed in light of the previous finding that there is a similar increase in FFA levels in females (51). However the possibility of contamination by cross-reacting prolactin in the immunochemical assay has not been ruled out. The recent observation that testosterone may play a lipolytic role *in vivo* (42), may explain the observation that post-pubertal males have a significantly smaller amount of subcutaneous fat than females.

The physiological role of the other pituitary peptides and glucagon as previously discussed is even more tenuous and requires further study.

5. SUMMARY AND CONCLUSIONS

The metabolism of adipose tissue is outlined and the interrelationships of glucose and fatty acids discussed.

The hormonal and neural control of fat mobilization are discussed and the metabolic consequences outlined. The metabolic changes in starvation and ketosis are reviewed in the light of more recent work.

A physiological role of hormones is discussed in relation to the metabolic regulation during exercise, starvation, exposure to cold, growth of immature animals and "stress". Insulin and glucose appear to exert their effect on adipose tissue metabolism as follows: a) they control the rate of lipogenesis, b) they influence FFA release through their effect on reesterification and c) by their action on cerebral metabolism they may control the secretion of growth hormone and help to regulate sympathetic nervous activity. It appears likely that the sympathoadrenal system functions as a rapid and short-acting fat-mobilizing mechanism, while the pituitary provides for a slower and more sustained response which may be modified during prolonged starvation by a ketone-insulin feedback mechanism. The role of glucagon and of other pituitary lipolytic substances

is at present uncertain. A permissive role in fat mobilization has been ascribed to the thyroid and adrenal cortex.

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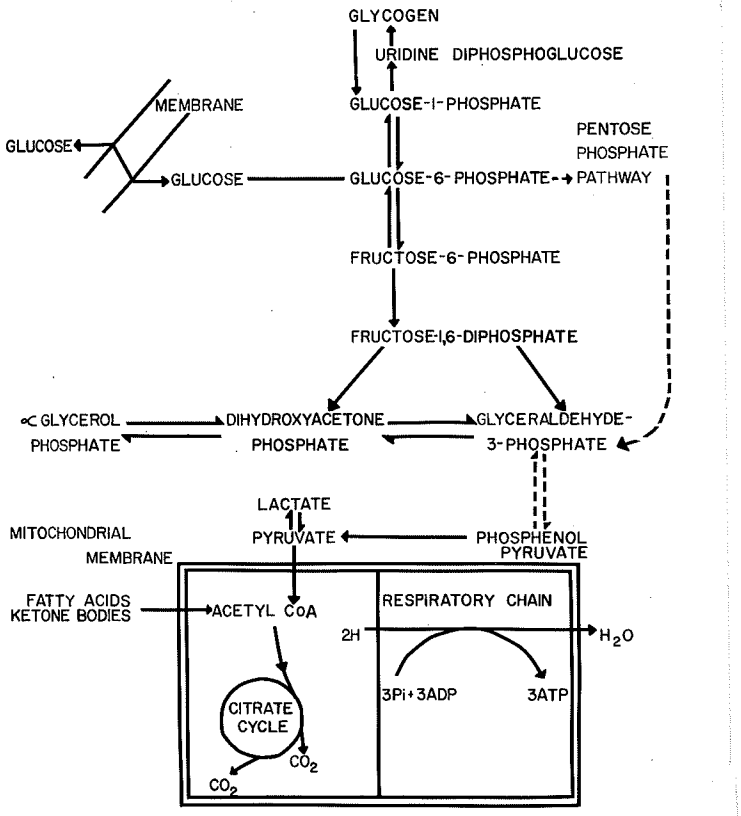

FIGURE 1 Glucose, fatty acid and energy metabolism.

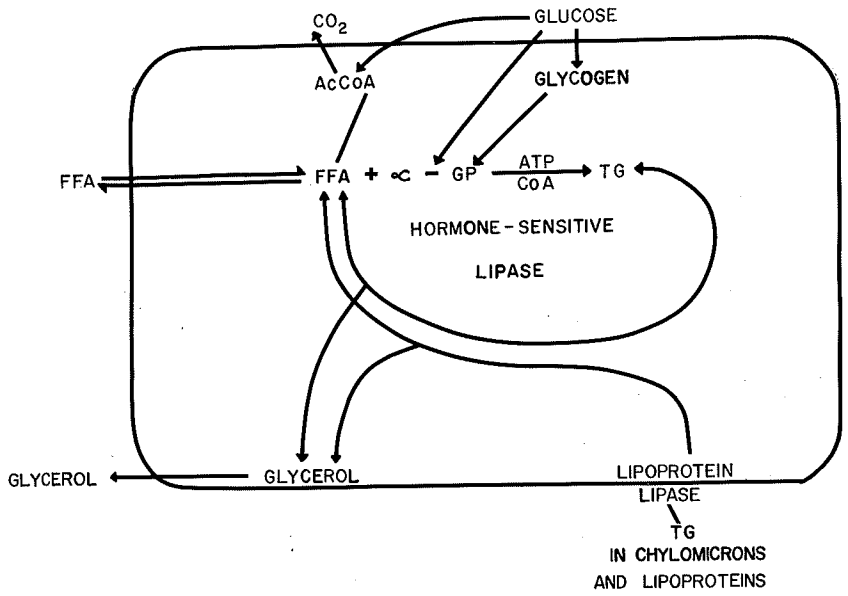

FIGURE 2 Schematic representation of an adipose tissue cell indicating the major input and output pathways for fat deposition and mobilization.

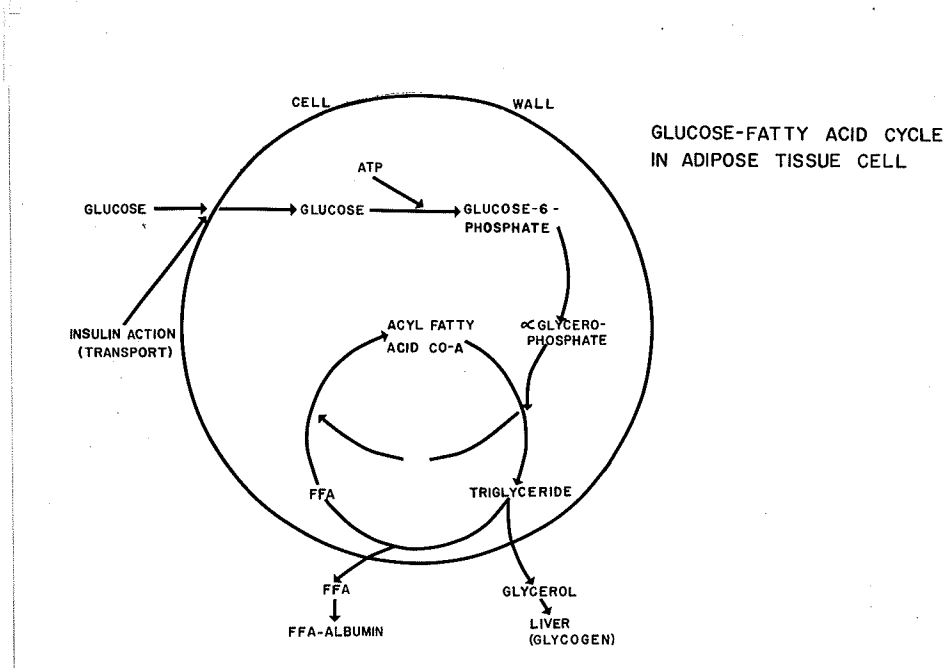

FIGURE 3 The glucose-fatty acid cycle as proposed by Randle et al (27).