Glucose Metabolism in Normal and Obese Subjects

Effect of Phenformin

Robert A. Kreisberg, M.D., Birmingham

SUMMARY

The purpose of the experiments described in this paper was to determine whether phenformin altered the metabolism of glucose in subjects in whom it did not lower the blood sugar. Glucose-1-C-14 was utilized to investigate the metabolism of glucose in eight normal and eight obese subjects before and after phenformin.

Phenformin increased the turnover, recycling and conversion of glucose to lactate and decreased the oxidation of glucose in all of the normal subjects studied. The increase in glucose turnover was due entirely to the increase in glucose recycling since net glucose turnover (glucose derived from all sources except the Cori cycle) did not change. Phenformin also increased glucose turnover and recycling in obese subjects but in contrast to normals, its effects on glucose oxidation and glucose conversion to lactate were more variable.

These results establish that phenformin has clearly defined effects on the metabolism of glucose in subjects in whom it exerts no hypoglycemic action and support the concept that phenformin increases glucose utilization by diverting it from oxidative to nonoxidative pathways in peripheral tissues. DIABETES *17*:481-88, August, 1968.

Despite many years of intensive investigation, the mechanism of phenformin's action in diabetes is still not understood. Although it is unlikely that the results of studies performed in animals are applicable to man, it has been assumed that phenformin lowers the blood sugar of diabetic subjects by increasing peripheral glucose utilization.[1] Studies of the effect of phenformin on glucose metabolism in humans have been infrequent and the results conflicting. Butterfield, Fry and Holling[2] demonstrated an increase in peripheral glucose uptake in diabetic patients responding to phenformin. Madison and Unger[3] were unable, however, to confirm these observations in diabetic patients in whom phenformin also effectively lowered the blood sugar. Of great interest recently is the demonstration by Searle and co-workers[4] that phenformin increased glucose oxidation by diabetic subjects.

One of the more intriguing aspects of this problem has been the apparent lack of effect of phenformin in normal subjects. Its failure to lower blood sugar levels[5] has carried the implication that the metabolism of glucose was unaltered. Recent studies of glucose kinetics in normal subjects treated with phenformin, however, revealed that such an assumption was probably incorrect. As a result of changes observed in blood glucose specific activity decay curves, Searle and co-workers[6] have suggested that phenformin increases Cori cycle activity and glucose synthesis in both normal and diabetic subjects. They have speculated that the difference in blood sugar responses are due to the ability of the normal subject to make certain metabolic adjustments to prevent hypoglycemia which the diabetic patient is unable to do, rather than to any difference in the primary mechanism of action of phenformin.

The following studies were designed to investigate the effects of phenformin on glucose turnover and oxidation in human subjects with technics which would also allow direct measurement of glucose recycling.

METHODS

Subjects: Eight normal and eight obese subjects were utilized to investigate the effects of phenformin on glucose metabolism. All subjects were placed on American Diabetes Association diets for fourteen days which were calculated to maintain a constant body weight. Glucose turnover studies were performed on day 7 and on day 14, after the administration of 100-150 mg. of phenformin orally for seven days. Each subject received 50 mg. of phenformin orally ninety minutes before the turnover study on day 14. Glucose tolerance was established by the oral administration of 100 gm. or 1.75 gm. of glucose per kilogram body weight to each subject. Each subject was fully advised to the nature and extent of the study before consent was obtained.

Glucose turnover studies: 50-75 μc. of glucose 1-C-

The University of Alabama in Birmingham, The Medical Center, Birmingham, Alabama.

14* was administered over a period of two minutes to each subject through the tubing of an infusion of 0.9 per cent saline. Blood samples were withdrawn at thirty-minute intervals for four hours and deproteinized by the method of Somogyi.[7] Glucose was separated from the acidic components of the protein-free supernatant on an Amberlite MB-3† resin column in the bicarbonate form.[8] An aliquot of the eluate containing glucose was assayed directly for radioactivity in a liquid scintillation counting system. Glucose in another aliquot of the eluate was oxidized with periodate and the formaldehyde, derived from carbon atom 6, was isolated as the formaldimedone and counted in a liquid scintillation system.[9,10] Glucose recycling was determined from the blood glucose specific activity and the radioactivity in the formaldimedone derivative of glucose as described by Reichard et al.[10] Acidic components of the protein-free blood extract were eluted from the Amberlite MB-3 column with 0.1N NaCl and assayed for lactate concentration and radioactivity content. All radioactivity in this fraction was assumed to reside in lactate.‡ Although lactate may be distributed in total body water the per cent of the administered C-14 recovered in lactate in these experiments was determined by assuming a space of distribution equal to the glucose space, since the latter was measured directly in each study.

The glucose and lactate concentrations before and after phenformin represent the mean of eight individual determinations performed on blood samples obtained during the course of the turnover studies. Glucose and lactate were measured enzymatically.[11] Deproteinization of blood by the method of Somogyi resulted in a 25 per cent loss in lactate measured enzymatically, however, no correction factor was introduced.

Absolute glucose turnover (G^T) in the text and the figures refers to glucose turnover uncorrected for recycling and is the value that would be obtained if none of the radioactivity in the glucose disappearing were ultimately recycled to glucose. Net glucose turnover (G^{T-R}) is the absolute glucose turnover corrected for recycling and it represents glucose derived from all sources except glucose itself. It is comparable to the values obtained with technics utilizing randomly labeled glucose. Under the steady state conditions imposed in these studies absolute and net turnover rates were assumed also to represent absolute and net rates of glucose synthesis. The contribution of glycogen breakdown to glucose turnover was minimized by fasting each subject twelve to fourteen hours before study.

Glucose oxidation was determined by the method of Manougian.[12] Expired air was collected for two minutes at thirty-minute intervals for four hours and the minute volume was measured by use of a Wright physiologic respirometer. Samples were analyzed immediately for CO_2 content with a Model LB-1 Medical Gas Analyzer* and C-14-O_2 was trapped in Hyamine[13] for subsequent liquid scintillation counting.

A Beckman ambient temperature liquid scintillation spectrometer with an external standard was used for all counting. Counting efficiency was checked routinely with C-14-toluene and C-14-glucose internal standards. The formaldimedone derivative of glucose and the Hyamine samples containing C-14-O_2 were counted in a toluene-PPO solution, while aqueous samples were counted in a dioxane-naphtholene-PPO solution.

Calculations and analysis of the glucose and CO_2 specific activity curves were accomplished with the aid of an IBM 7040 computer.

RESULTS

The pertinent clinical information is presented in table 1. The per cent deviation of subjects from their ideal body weight ranged from —8 to +12 per cent for normals and +19 to 87 per cent for obese subjects. There was a family history of diabetes in three of the normal subjects but only in one of the obese subjects. There was a strong family history of obesity in the obese group. Therapy with phenformin did not significantly alter body weight in the normal or obese groups.

Normal subjects: The alterations produced by phenformin in the metabolism of glucose by a single normal subject are shown in figure 1. The changes illustrated are representative of those observed for the normal group as a whole. The blood glucose concentration did not change following phenformin. Absolute glucose turnover (G^T) increased 30 mg./kg./hr., glucose recycling (G^R) increased 31 mg./kg./hr. and, as a result, net glucose turnover (G^{T-R}) remained unchanged. Glucose oxidation (G^{CO2}) decreased 33 mg./kg./hr. Divergence of the zero time intercepts in this subject was not due to differences in glucose pool size but to dif-

*New England Nuclear Corporation, Boston, Mass.

†Rohm and Hass Company, Philadelphia, Pa.

‡It was determined by gradient formic acid elution from a Dowex 1-x8 resin column in the formate form that over 95 per cent of the radioactivity in this fraction resided in lactate.

*Beckman Instrument Company, Spinco Division, Chamblee, Ga.

TABLE 1

Clinical data for normal and obese subjects

Subject	Sex	Age yrs.	Weight Initial kg.	Weight DBI kg.	Height inches	Per cent deviation from ideal body weight*	Family history Diabetes	Family history Obesity	Blood glucose Fasting	Blood glucose 2-hr.
Normals										
CB	F	29	50.0	48.6	62.0	− 8	0	0	73	51
JMi	M	34	75.0	74.6	70.5	0	0	0	73	74
JB	F	22	62.7	62.3	65.5	+ 4	0	0	64	90
PT	F	26	64.2	66.1	70.0	− 6	0	0	68	60
MR	F	27	62.0	62.0	63.0	+ 9	0	0	57	80
VW	F	26	61.0	61.0	67.0	− 3	+	0	79	118
JMo	M	30	87.8	86.0	71.5	+12	0	0	79	72
PH	F	31	59.0	61.8	63.0	+ 6	+	+	70	80
Obese										
GS	F	25	70.5	70.2	65.0	+19	0	0	66	101
MW	F	19	89.5	89.5	64.0	+59	0	0	71	72
VP	F	44	121.0	120.0	65.0	+87	0	+	72	140
RL	F	43	96.2	96.0	63.5	+51	0	0	76	92
JG	M	23	107.8	107.3	72.0	+26	+	+	76	112
DC	F	38	84.5	84.0	66.5	+28	0	+	75	85
CM	F	28	72.0	72.5	62.5	+26	0	+	74	102
JA	M	23	86.3	85.3	67.0	+33	0	+	71	106

*From Metropolitan Life Insurance Tables 1959

ferences in the amount of glucose-1-C-14 administered as a tracer.

The values for the various parameters of glucose metabolism following phenformin are expressed as a per cent of the control value in figure 2. The pattern of response was remarkably uniform. Absolute glucose turnover increased in seven of eight normal subjects studied. Glucose recycling and glucose conversion to lactate increased in all of the subjects while glucose oxidation decreased in all of the normal subjects. No change was observed in the mean blood glucose concentration, the size of the glucose pool or in CO_2 production but blood lactate concentration increased significantly (table 2). Paired comparison analysis (Student's *t*-test) revealed that the increase in absolute glucose turnover, glucose recycling and glucose conversion to lactate and the decrease in glucose oxidation following phenformin were highly significant. The increase in absolute glucose turnover and the increase in glucose recycling, expressed as mg./kg./hr., were balanced so that net glucose turnover was not altered (table 2). It is of interest that the mean decrease in glucose oxidation was almost identical to the mean increase in absolute glucose turnover (19 vs. 22 mg./kg./hr.). This suggests that the substrate for the increase in absolute turnover was derived from the glucose which was diverted from oxidative pathways into lactate.

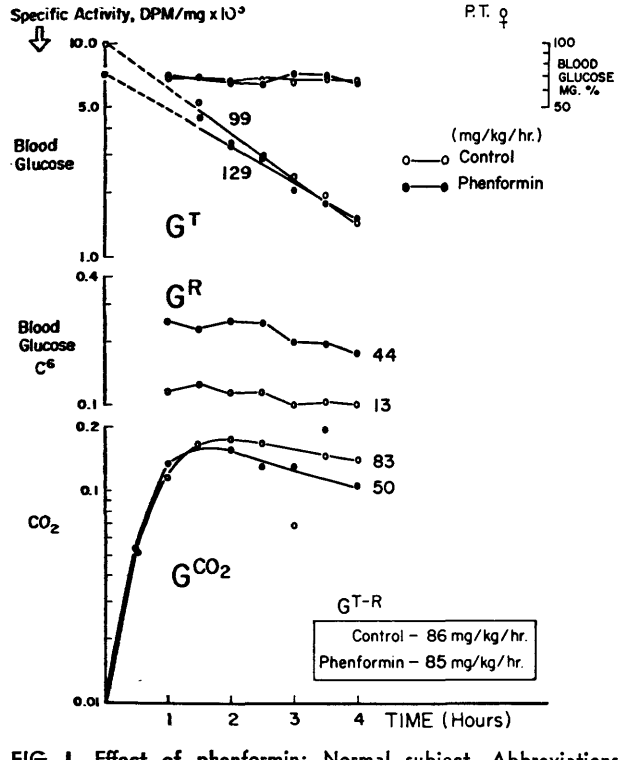


FIG. 1. Effect of phenformin: Normal subject. Abbreviations used are: G^T (absolute glucose turnover rate), G^R (glucose recycle rate), G^{CO_2} (glucose oxidation rate), and G^{T-R} (net glucose turnover rate).

Obese subjects: The changes produced by phenformin in the glucose metabolism of obese subjects were less uniform than those observed in the normals, particularly in regards to oxidation of glucose and its conversion to lactate. Studies of glucose metabolism before and after phenformin in two obese subjects demonstrating dif-

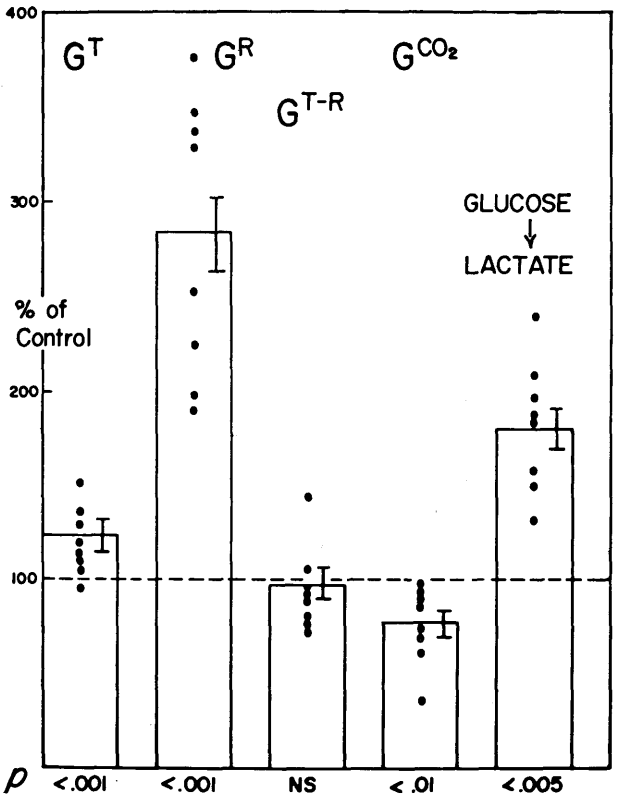


FIG. 2. Per cent change in glucose metabolism produced by phenformin in normal subjects. The height of the bars represents the mean ± S.E.M. p determined by paired comparison analysis and Student's t-test.

ferent types of responses to phenformin are shown in figures 3 and 4. Subject V. P., shown in figure 3, represents a pattern of response observed in four obese subjects. Absolute glucose turnover and glucose recycling increased by comparable amounts, 17 and 15 mg./kg./hr. respectively, so that net glucose turnover remained unchanged. In contrast to the decrease observed in normals and the four other subjects, glucose oxidation increased in this and three other obese subjects. A different pattern of response, characterized by that of C.M. and shown in figure 4, was observed in the other four obese subjects. Absolute glucose turnover following phenformin did not change or increase to a lesser extent than did glucose recycling so net glucose turnover decreased. In subject C.M., glucose recycling doubled so that net glucose turnover decreased by 10 mg./kg./hr. Glucose oxidation decreased in this subject, by 11 mg./kg./hr., and in the three other subjects, thus resembling the response observed in the normals.

The changes produced in glucose metabolism by phenformin in the obese group of subjects are shown in figure 5 and are expressed as a per cent of the control value. The increase in absolute glucose turnover and in glucose recycling is similar to that observed in normal subjects. Absolute glucose turnover increased or stayed the same in seven subjects while glucose recycling increased in all eight subjects with a mean value that was 185 per cent of the prephenformin value. In contrast to the normal group, however, the oxidation of glucose and its conversion to lactate following phenformin were more variable, decreasing in four and three subjects respectively. When the individual values following phenformin were expressed as a per cent of the control value and analyzed by paired comparison, only the change in glucose recycling was significant. This is also demonstrated in table 2 where mean values before and after phenformin are presented. In contrast to the negative correlation between glucose oxidation and lactate formation observed in normal subjects following phenformin those obese subjects demonstrating the increase in oxidation were also the ones in whom glucose conversion to lactate increased. The explanation for this incongruity is not clear. There were no clinical or laboratory features by which these groups of obese subjects could be distinguished.

Comparison of normal and obese subjects (table 2):

There was no difference between the control blood glucose and lactate concentrations of normal and obese subjects. Following phenformin, blood glucose concentrations did not change but lactate concentrations increased significantly in normal subjects. Resting CO_2 production was significantly less in normal than obese subjects before phenformin; but following phenformin CO_2 production decreased in obese subjects so that no difference remained. The glucose pool size of obese subjects prior to phenformin was significantly greater than that of the normals. Absolute glucose turnover and glucose recycling prior to phenformin were the same for normal and obese subjects. However, their response to the drug was quantitatively different. The increase in absolute glucose turnover and glucose recycling was significantly less than that of normal subjects.

DISCUSSION

The results of the current studies clearly demonstrate that phenformin alters the metabolism of glucose in subjects in whom no hypoglycemic effect is observed. Although the changes produced by phenformin in the over-all metabolism of glucose by obese subjects were more variable than those seen in normals, the changes in glucose recycling and absolute turnover were remarkably similar for both groups. Glucose recycling in-

TABLE 2
Effects of phenformin (DBI)

	DBI	Normal (8)*	Obese (8)	p‡
Blood glucose	−	70±2†	73±1	NS
mg. per 100 ml.	+	69±2	72±2	NS
	p§	NS	NS	
Blood lactate	−	0.699±.070	0.738±.066	NS
μmoles/ml.	+	1.019±.088	0.860±.055	NS
	p	<.001	NS	
CO_2 production	−	413±25	540±45	<.05
mg./min.	+	416±31	460±23	NS
	p	NS	NS	
Glucose pool	−	16.35±1.27	21.55±1.63	<.05
gms.	+	17.58±1.19	19.42±1.07	NS
	p	NS	NS	
Absolute glucose turnover	−	118±4	108±8	NS
mg./kg./hr.	+	140±9	115±6	<.02
	p	<.02	NS	
Glucose recycling	−	13.4±0.7	15.0±0.7	NS
per cent	+	31.1±1.9	25.1±1.8	<.05
	p	<.001	.005	
Glucose recycling	−	15.8±1.0	15.8±1.0	NS
mg./kg./hr.	+	43.3±2.7	28.6±2.4	<.005
	p	<.001	.005	
Net glucose turnover	−	102±3	92±7	NS
mg./kg./hr.	+	101±8	87±5	NS
	p	NS	NS	
Glucose to lactate	−	2.56±0.20	3.41±0.29	<.05
per cent	+	4.17±0.23	3.42±0.36	NS
	p	<.005	NS	
Glucose oxidation	−	77±9	44±6	<.02
mg./kg./hr.	+	58±8	49±8	NS
	p	<.01	NS	
Glucose turnover oxidized	−	66±7	43±7	<.05
per cent	+	42±6	45±9	NS
	p	<.005	NS	

*Number of subjects.
†All values represent mean ± S.E.M.
‡Normal vs. obese (Student's *t*-test).
§ − vs. + (paired comparison analysis—Student's *t*-test).

creased in all of the normal and obese subjects studied and absolute glucose turnover increased in fourteen of the sixteen subjects. The increase in absolute glucose turnover was solely due to the effect of phenformin on glucose recycling since net glucose turnover, that is, glucose derived from all sources except recycled glucose, remained unchanged. An apparent increase in glucose recycling could have been observed as a result of a decrease in the size of the 3 carbon precursor pool of glucose, such as would have occurred if phenformin had inhibited deamination of amino acids.[14] This did not occur, however, since net glucose turnover would have decreased instead of remaining unchanged. Furthermore, the increased recovery of C-14 from glucose in lactate and the increase in lactate pool size (glucose space × lactate concentration) observed in the normals supports the contention that phenformin increased glucose recycling by enhancing peripheral conversion of glucose to lactate. These results, therefore, confirm the original suggestion made by Searle and coworkers[6] and support their proposal that phenformin does not produce hypoglycemia in the nondiabetic subject because enhanced peripheral disposal of glucose is balanced by increased glucose synthesis. They have speculated that the ability of the diabetic to increase glucose synthesis is limited in some way so that enhanced glucose disposal is not accompanied by increased glucose synthesis and blood sugar levels decline. The alterations produced by phenformin in the metabolism of glucose by obese subjects may have some bearing on this, although there was no lowering of the blood sugar. The increase in glucose recycling was significantly less in the obese subject than in the normal (table 2; $p < .005$). This was a true difference since

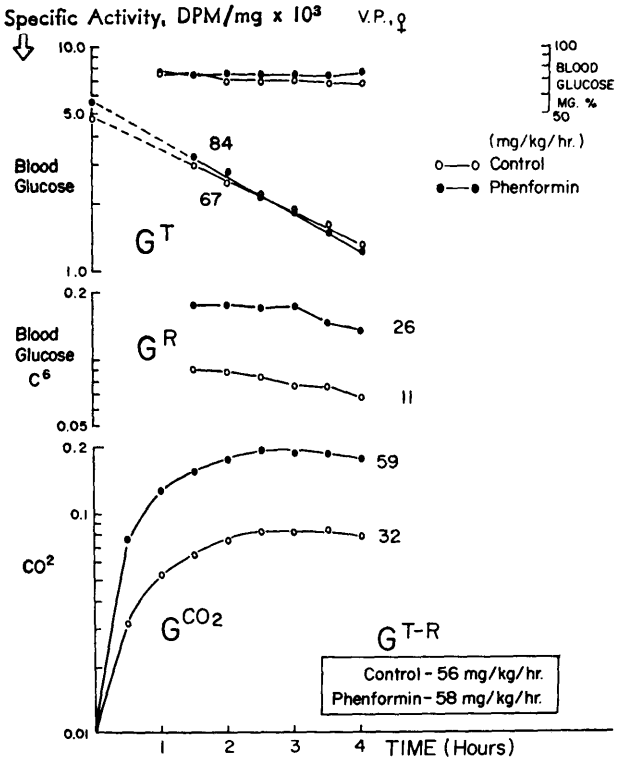
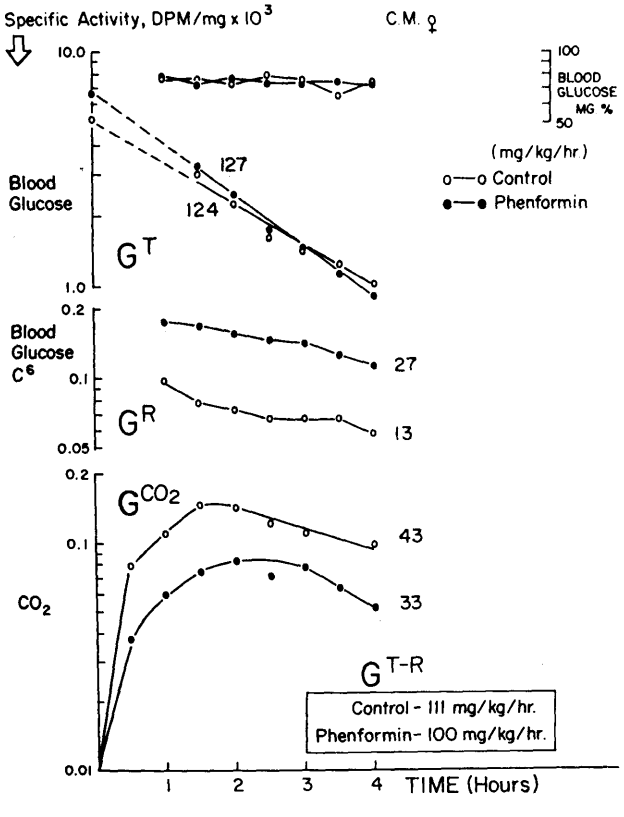


FIG. 3. Effect of phenformin: Obese subject.



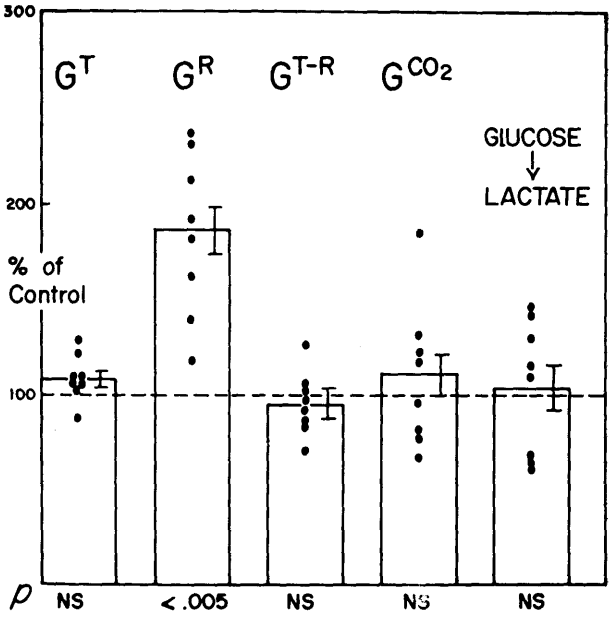


FIG. 5. Per cent change in glucose metabolism produced by phenformin in obese subjects.

recycling is calculated as a per cent of the absolute turnover and there was no difference between the pre-phenformin values of obese and normal subjects. Thus, there is suggestive evidence in subjects, who as a group share some of the metabolic features of maturity-onset diabetes, that the ability to respond to phenformin by an increase in glucose recycling may be limited.

In direct contrast to the observations of Searle and co-workers[4] in diabetic subjects, phenformin decreased glucose oxidation in all of the normal and half of the obese subjects in this study. The combination of decreased glucose oxidation and increased conversion of glucose to lactate in the normal subjects indicates that phenformin alters the intracellular fate of glucose and it is compatible with the concept that its effect on peripheral glucose metabolism is mediated through enhanced anaerobic glycolysis. This may not be the only explanation, however, since total CO_2 production in normal subjects did not change after phenformin. This means that the decreased oxidation of glucose must have been accompanied by increased oxidation of alternate fuels. It is unlikely that circulating lipids could provide the extra substrate necessary for oxidation since plasma cholesterol and triglyceride concentrations are reduced by phenformin.[15] Although there are no studies on the effects of phenformin on plasma free fatty acid levels, one could conclude by extrapolation from its

FIG. 4. Same as figure 5.

effects on other blood lipids, that they were also decreased. However, neither the mechanism by which phenformin enhances peripheral disposal of glucose nor the identity of the alternate fuels for oxidation is important in regard to the theory of Searle and co-workers. The reason for the discrepancy between these results and those of Searle is not clear but may be due to differences in types of subjects studied or in the isotopes (they used randomly labeled glucose) or technics used for measuring C-14-O_2 production (they used a continuous CO_2 monitoring system). Unfortunately they did not determine glucose oxidation in their normal subjects so direct comparison of results is not possible. On the other hand, it is possible that these results may not be mutually exclusive. If glucose oxidation via the hexose monophosphate shunt was diminished by phenformin more glucose could be made available for direct oxidation thus accounting for the increase observed in studies with randomly labeled glucose and the decrease in studies with glucose-1-C-14. Abnormal glucose tolerance was present in only one of the obese subjects in whom glucose oxidation increased so that the presence or absence of diabetes did not correlate, in this small series, with the effects of phenformin on glucose oxidation.

Since phenformin is not uniformly effective in lowering the blood sugar in all maturity-onset diabetics[5] it may be reasonable to expect that it would also have different effects on the metabolism of glucose in subjects who appear, at least superficially, to represent a homogeneous group. For example, it may not simply be the presence of carbohydrate intolerance which determines the response of the patient to phenformin but whether or not the intolerance is associated with elevated, normal or less than normal levels of insulin. The studies of Pereira and co-workers[16] are pertinent in this regard since they have demonstrated that phenformin only alters glucose disposal in normal subjects when glucose and insulin levels are both increased, such as occur after a glucose tolerance test or with the infusion of glucose and insulin. Phenformin does not potentiate or augment the effects of insulin administered by itself. Since insulin levels were not measured in this series of obese subjects, it is not possible to correlate the metabolic changes produced by phenformin and the insulin secretory response to glucose.

The findings of Berger and co-workers[17] that phenformin reduced the blood sugar and insulin levels of subjects treated with corticosteroids and the recent in vitro observations that phenformin inhibited gluconeogenesis in the perfused guinea pig liver,[18] at concentrations that are therapeutically effective in humans, suggested again, that the ability of this drug to lower blood sugar and insulin levels could just as reasonably be explained by inhibition of glucose production as enhanced utilization. Previous studies concerning the effects of phenformin on hepatic glucose release in humans have been inconclusive. Beringer and co-workers[19] observed decreased glucose release from the liver by hepatic vein catheterization. Tranquada and co-workers,[20] however, using comparable technics were unable to confirm these findings. In steady state situations in which blood sugar concentrations remain constant, glucose utilization is balanced by glucose replacement so that the glucose turnover reflects the rate of glucose synthesis. Following phenformin, net glucose synthesis did not change indicating that the increase in absolute glucose synthesis was due only to increased recycling and that the contributions made by glycogen, amino acids and other 3 carbon precursors of glucose remained unchanged. Increased recovery of glucose-C-14 in lactate and increased glucose recycling indicate that the elevation of blood lactate levels following phenformin,[21] observed in normal subjects in these studies, are not due to impaired incorporation of lactate into glucose such as might occur with inhibition of gluconeogenesis but is due to increased peripheral conversion of glucose to lactate. Therefore, though appealing, there is no evidence in normal or obese subjects that phenformin inhibits gluconeogenesis as I proposed in preliminary communications.[22,23] However, since none of these subjects were diabetic and none developed hypoglycemia, such a mechanism of action has not been totally excluded.

Although glucose turnover has been studied extensively in humans, there have been relatively few studies of glucose recycling. The limited information that is available on this aspect of glucose metabolism in humans is a result of the technic described by Reichard and his co-workers, however, less than forty subjects have been studied.[9,10,24,25] Glucose recycling represented 16 per cent of glucose turnover in normal subjects, 24 per cent of glucose turnover in fasted normal subjects, 27 per cent of glucose turnover in patients with cancer, and 35 per cent of glucose turnover in obese subjects. Since the total number of subjects in each group was small and the range of variation great, these were not significantly different values. The results of the present study, therefore, are comparable to those obtained by these investigators. Although it has been

proposed that gluconeogenesis may be increased in obesity,[22,26,27] there was no evidence that such was the case when the normal and obese subjects investigated in this study were compared. The only difference in the metabolism of glucose by normal and obese subjects was the observation, previously made by others, that glucose oxidation was decreased in obesity.[28] The variability of the response of the obese subject to phenformin should not be surprising in view of the multitude of metabolic defects that have been described in obesity[29] and it strongly re-emphasizes that obesity may be a heterogeneous disorder with two or more subgroups.

ACKNOWLEDGMENT

This study was supported by United States Public Health Service Grants AM 09722-03 and 2MOI FR-32-08, and USV Pharmaceutical Company.

The excellent technical assistance of Gail Yerby, Anne Stefanu, James Gleaves, Brent McFarland, Bert Harwell and Lawrence Farris is gratefully acknowledged. I would also like to thank Dr. George A. Reichard and Mr. Gus Stefanu for helpful advice with both the methods and the computer program.

REFERENCES

1 Grodsky, G. M., Karam, J. H., Pavlatos, F. C., and Forsham, P. H.: Reduction by phenformin of excessive insulin levels after glucose loading in obese and diabetic subjects. Metabolism *12*:278-86, 1963.

2 Butterfield, J., Fry, I. K., and Holling, E.: Effects of insulin, tolbutamide and phenethyldiguanide on peripheral glucose uptake in man. Diabetes *7*:449-54, 1958.

3 Madison, L. L., and Unger, R. H.: Effect of phenformin on peripheral glucose utilization in human diabetic and nondiabetic subjects. Diabetes *9*:202-06, 1960.

4 Searle, G. L., and Cavalieri, R. R.: Glucose kinetics before and after phenformin in the human subject (to be published in a forthcoming issue of the New York Academy of Sciences).

5 Fajans, S. S., Moorhouse, J. A., Doorenbos, H., Lovis, L. H., and Conn, J. W.: Metabolic effects of phenethylbiguanide in normal subjects and diabetic patients. Diabetes *9*:194-201, 1960.

6 Searle, G. L., Schilling, S., Porte, D., Barbaccia, J., DeGrazia, J., and Cavalieri, R. R.: Body glucose kinetics in nondiabetic human subjects after phenethylbiguanide. Diabetes *15*:173-78, 1966.

7 Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69-73, 1945.

8 Friedman, B., Goodman, E. H., and Weinhouse, S.: Dietary and hormonal effects on gluconeogenesis in the rat. J. Biol. Chem. *240*:3729-35, 1965.

9 Cahill, G. F., Herrera, M.D., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A., and Kipnis, D. M.: Hormone-fuel interrelationships during fasting. J. Clin. Invest. *45*:1751-69, 1966.

10 Reichard, G. A., Moury, N. F., Hochella, N. J., Patterson, A. L., and Weinhouse, S.: Quantitative estimation of the Cori cycle in the human. J. Biol. Chem. *238*:485-501, 1963.

11 Bergmeyer, H. U.: Methods of Enzymatic Analysis. New York, Academic Press, 1965.

12 Manougian, E.: Development of a single tracer injection method for C-14 glucose kinetic studies in humans. J. Nucl. Med. *5*:746-62, 1964.

13 Fredrickson, D. S., and Ono, K.: An improved technique for assay of C-14-O_2 in expired air using the liquid scintillation counter. J. Lab. Clin. Med. *51*:147-51, 1958.

14 Steiner, D. F., and Williams, R. H.: Actions of phenethylbiguanide and other compounds. Diabetes *8*:154-57, 1959.

15 Schwartz, M. J., Mirsky, S., and Schaefer, L. E.: The effect of phenformin hydrochloride on serum cholesterol and triglyceride levels of the stable adult diabetic. Metabolism *15*:808-22, 1966.

16 Pereira, V. G., Wajchenberg, B. L., and Shnaider, J.: Mechanism of action of phenethylbiguanide in normal subjects. Diabetes *16*:302-05, 1967.

17 Berger, S., Downey, J. L., and Traisman, H. S.: Reversal by phenformin of the contra-insulin effect of cortisone. Presented at the 49th Meeting of the Endocrine Society, Americana Hotel, Bar Harbour, Florida, June 15, 1967.

18 Altschuld, R. A., and Kruger, F. A.: Inhibition of hepatic gluconeogenesis in guinea pig by phenformin (to be published in a forthcoming issue of The New York Academy of Science).

19 Beringer, A., Hupka, K., Mosslacher, K., Moser, K., and Wenger, R.: Zur Beeinflussung des menschlichen Diabetes mit Insulin, blutzuckersenkenden Sulfonamiden sowie den Biguaniden. Wein. Med. Wschr. *108*:639-43, 1958 (quoted by Creutzfeldt, W., and Soling, H. D., Oral treatment of diabetes-A clinical and experimental review. Berlin, Springer-Verlag, 1961).

20 Tranquada, R. C., Kleeman, C., and Brown, J.: Some effects of phenethylbiguanide on human hepatic metabolism as measured by hepatic vein catheterization. Diabetes *9*:207-14.

21 Craig, J. W., Miller, M., Woodward, H., and Merik, E.: Influence of phenethylbiguanide on lactic, pyruvic and citric acids in diabetic patients. Diabetes *9*:186-93, 1960.

22 Kreisberg, R. A.: Kinetics of glucose utilization in obesity. Effect of phenformin. Ann. N. Y. Acad. Sci. *148*:743-54, 1968.

23 Kreisberg, R. A.: Inhibition of gluconeogenesis in humans by phenformin. Clin. Res. *16*:50, 1968.

24 Reichard, G. A., Moury, N. F., Hochella, N. J., Putnam, R. C., and Weinhouse, S.: Metabolism of neoplastic tissue, XXVI. Blood glucose replacement rates in human cancer patients. Cancer Res. *24*:71-76, 1964.

25 Rabinowitz, J. L., and Meyerson, R. M.: The effects of triiodothyronine on some metabolic parameters of obese individuals. Metabolism *16*:68-75, 1967.

26 Kreisberg, R. A., Boshell, B. R., DiPlacido, J., and Roddam, R. F.: Insulin secretion in obesity. New Eng. J. Med. *276*:314-19, 1967.

27 Arky, R. A., and Freinkel, N.: Alcohol hypoglycemia. V. Alcohol infusion to test gluconeogenesis in starvation with special reference to obesity. New Eng. J. Med. *274*:426-33.

28 Gordon, E. S., and Goldberg, M.: Studies of energy metabolism in human subjects using carbon 14-labeled compounds. I. Effect of sex, state of nutrition and body weight. Metabolism *13*:775-90, 1964.

29 Gordon, E. S., Goldberg, M., and Chosy, G. J.: A new concept in the treatment of obesity. JAMA *186*:50-60, 1963.