Effects of Growth Hormone on the Transport of Lipids in Blood

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The view is widely held that the forms in which lipids are carried in the blood may be of importance in atherosclerosis and related disorders.[1] A comparative study of lipid transport under the conditions of mobilization of fat from the body depots and of absorption of fat from the intestine was therefore undertaken. Mobilization was induced by fasting and by the administration of purified growth hormone. Fat absorption, with ensuing movement of lipid towards the depots, was obtained by feeding a fat meal. Lipid transport was also studied under conditions of increased fat utilization in the prediabetic state caused by repeated injections of growth hormone in regularly fed dogs. The results demonstrate marked differences in the amounts and characteristics of the lipids transported in blood under these conditions.

METHODS, MATERIALS AND EXPERIMENTAL PROCEDURES

Adult dogs of either sex were kept in metabolism cages in air-conditioned rooms. Venous blood samples were collected with heparin as anticoagulant for determinations of sugar, erythrocyte sedimentation rate and volume per cent of corpuscles and of plasma by methods given previously.[2] Blood samples were allowed to clot for one hour at 4° C. The optical density and the unesterified fatty acids (UFA) of the sera were determined according to Grossman, Stadler, Cushing and Palm.[3] Values for blood lipids were obtained by a slight modification of Bloor's alcohol-ether extraction method.[4] The alcohol-ether extracts were taken to dryness in vacuo (water-bath 45°); lipids in the residue were taken up in 3:1 (v/v) petroleum ether (b.p. 40-60°)—chloroform. Cholesterol was determined on aliquots of the petroleum ether-chloroform solutions by the method of Sperry and Webb.[5] Phospholipid P was determined by King's method[6] and phospholipid was calculated using the factor 25.0 x P. Total lipids were determined gravimetrically on aliquots of the petroleum ether-chloroform mixture after evaporation of the solution. No total fatty acid and cholesterol ester determinations were carried out which would allow for accurate calculation of serum triglycerides. A rough estimation, however, of the serum triglyceride level may be arrived at by subtracting the values for phospholipids and total cholesterol from the values for total lipids.[7-9] Approximately two thirds of this difference may be regarded as "neutral fat," the remaining one third being due to cholesterol ester fatty acid and unesterified fatty acid (UFA or NEFA). The serum lipoprotein fractions were estimated by the ultracentrifugal technic of Lewis, Green and Page.[10] The —S 20-40 and —S 1-20 fractions are referred to as β and α lipoproteins, respectively. The estimation of the lipoproteins at d 1.21 does not permit measurement of the extremely low density chylomicrons. For a determination of this component optical density has been used. The amount of blood required for all these determinations was approximately 35 ml. These large withdrawals of blood were tolerated without apparent changes. The times of the blood withdrawals are indicated in figure 1. In the experiment on fasting (figure 1, column 1) the first blood sample was withdrawn six hours postprandially; in the experiment on fat feeding prior to the test meal, which was given forty-two hours after the last food intake (column 2). When growth hormone was injected into fasting animals, the first blood sample was taken forty-two hours postprandially as well (figure 1, column 3). In the dogs fed twice daily and injected with growth hormone (column 4) the blood samples were taken in the "fasting" state prior to their morning meal, that is, eighteen hours after their last food intake on the previous day. *No postprandial* blood samples were taken from these growth-hormone injected, fed dogs. The experimental periods were separated by intervals of two to three weeks.

Previous to, and during the intervals between the

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Presented before the Canadian Federation of Biological Societies, Kingston, Ontario, June, 1958.

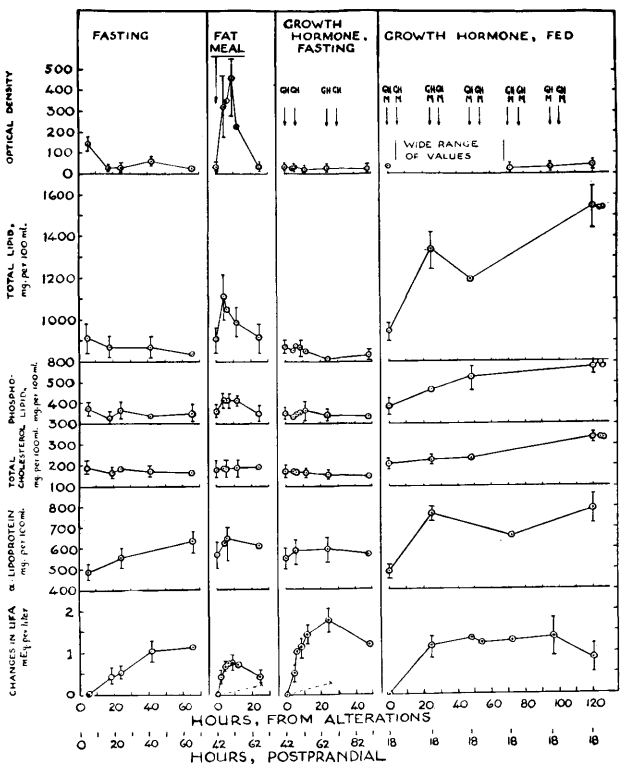

FIG. 1. Mean values and standard errors of blood lipids of nine dogs. G. H. indicates the injection of growth hormone, M the administration of a meal. The broken lines under columns "Fat Meal" and "Growth Hormone, Fasting" indicate the rises in UFA due to fasting alone. Note that the UFA are expressed as changes from the initial value.

experiments, the dogs were given meals, at 10 a.m. and 4 p.m., of horse meat in the amount of 1,430 calories per square meter of body surface per day. At all times water was available ad libitum. In the experiment on fat absorption the dogs were fasted for a day and on the following day at 10 a.m. (forty-two hours postprandial) were given the test meal of 10 gm. fat per kilogram of body weight, which was usually consumed within half an hour. In the case of two dogs (774 and 860) it was mixed with about 100 gm. of horse meat to increase its palatability. The fat fed was either lard, to dogs 471, 859 and 860, or fat rendered from dogs' adipose tissues, to dogs 771 and 774. In the experiment on fat mobilization the dogs were fasted for a day and on the following day were given subcutaneous injections of growth hormone at 10 a.m. (forty-two hours postprandial) and 4 p.m. The dosage of growth hormone, per kilogram of body weight per day, was 10 mg. to dogs 771 and 774, and 3 mg. to the others. In the feeding experiments 1.5 mg. of growth hormone (total dose daily 3 mg.) per kilogram of body weight was given subcutaneously at the time of feeding for five days. The purified, crystalline growth hormone (Campbell and Davidson),[11] Connaught Medical Research Laboratories, Lot 100, was generously provided jointly by the National Research Council of Canada, The National Cancer Institute and the Department of Health and Welfare. It was dissolved each day in saline at *p*H 8, 10 mg. per milliliter, for subcutaneous injection.

RESULTS

The mean results for the nine dogs used in these experiments are summarized in figure 1, and standard errors are indicated. As each animal served as its own control the consecutive values in each dog are more meaningful than is indicated by the standard errors. However, because of the mass of data, the individual values are presented for only one typical animal, dog 774 (table 1).

In the experiment on fasting animals, some effect of the last meal on optical density was evident at six hours, but from eighteen to sixty-six hours postprandial the serum remained clear. Following the fat meal the optical density rose sharply to a peak at eight hours; was still high at twelve hours but not at twenty-four hours. Much variation occurred in this response, both with respect to the time at which the peak occurred and to its height. When growth hormone was administered to the fasting dogs the optical density remained low. When growth hormone was given repeatedly for five days to fed dogs, the serum samples, taken eighteen hours postprandially, usually showed opacity within the first two days of injection, but by the third day had cleared. (Opacity of the serum during the first few days of injection of growth hormone has been noted in several other fed dogs in this laboratory. The serum then became clear despite repeated injections.)

The total lipids of the serum remained low under the conditions of fasting, and during the administration of growth hormone in the fasting state. They rose following the fat meal. The total lipids increased to the greatest extent when the dogs were fed and injected repeatedly with growth hormone; one day after the first injection the increase in lipids of the serum taken eighteen hours postprandially were definite, and after five days were still higher. The phospholipids of the serum did not change appreciably either during fasting, after the fat meal or when growth hormone was given during a period of fasting. However, a significant increase occurred when the dogs were fed and given growth hormone twice daily for five days. Likewise, the total cholesterol of the serum increased when the

TABLE 1

Serum lipids of a typically behaving dog (774) under various conditions

This dog was studied under the following conditions: (a) Fasting for 66 hr. (b) Given 42 hr. after the last food intake, a fat meal equivalent to 10 gm. per kg. of body weight. (c) Fasted for 42 hr. then given 10 mg. growth hormone per kg. of body weight per day during continued fasting for two days. (d) Regularly fed and injected twice daily with growth hormone, 3 mg. per kg. of body weight per day.

	Fasting					Fat meal					Growth hormone and fasting						Growth hormone and fed					
Lipid fraction Pp*	6	18	24	42	66	0	4	6	8	24	42	46	48	54	66	90	18	18	18	18	18	18
of serum Pi†											0	4	6	12	24	48	0	24	48	72	96	120
Optical density‡	155	29	29	38	22	25	495	393	260	28	39	24	49		32	22	19	160	112	25	37	32
Total lipids mg./ 100 ml.	1,009	992	816	775	814	988	1,259	1,181			889		876		809	830	1,040	1,205	1,370			1,623
Phospho-lipid mg./ 100 ml.	416	363	375	323	338	441	486	473			375		367		349	350	397	417	479			641
Total choles-terol mg./ 100 ml.	217	185	200	182	160	224	231	223			170		163		154	150	204	209	217			369
β-lipo-proteins (-S 20-40) mg./100 ml.		130	107	44	38	197	137	124			44		71		59	71	130	240				294
α-lipo proteins (-S 1-20) mg./100 ml.		480	527	535	640	527	564	633			535		595		610	625	420	720				862
Unesteri-fied fatty acids of serum mEq./L.	1.08	1.82	2.06	2.68	2.54	1.24	1.54	1.64	1.34	1.22	1.46	1.86	2.24	2.78	3.14	2.86	0.98	3.04	3.20	3.00	3.40	3.06

*Pp: — hours postprandial.
†Pi: — hours after first injection of growth hormone.
‡Optical density measured at 640 mμ.

dogs were fed and given growth hormone during five days; but did not change appreciably during the other experimental conditions.

The α-lipoprotein fraction (—S 1-20) increased significantly when the dogs were fed and given growth hormone. Likewise the β-lipoprotein fraction (—S 20-40) was affected significantly only under the condition of feeding plus growth hormone.

The UFA of the serum taken eighteen hours after feeding ranged from 0.82 to 1.83 mEq./L. This fraction increased during a fast and reached a plateau at about forty-two hours. The UFA rose slightly and insignificantly following the test meal, which was very high in fat and had very little or no carbohydrate or protein (see Methods). The most marked rise in UFA over the increase due to fasting alone, occurred in the dogs that were fasted and given growth hormone. This increase occurred rapidly with a peak at about twenty-four hours after first injection.* When growth hormone was given regularly with meals, the UFA of the eighteen-hour postprandial serum samples remained elevated during the five days of observation.

Hyperglycemia accompanied by glycosuria occurred in five of the nine dogs when they were fed and given growth hormone for five consecutive days, i.e., in Nos. 521, 647, 774, 471 (slight) and 759 (table 2). Hematocrit readings were obtained for each sample of blood withdrawn. For each of the various conditions to which the dogs were subjected, the final volume per cent of the plasma was either the same as or slightly above the initial value for that period. Confirming previous observations,[2] the erythrocyte sedimentation rate increased in fed dogs given growth hormone. During this period body weight increased by an average 6.9 per cent.

* This finding has been confirmed in man[20] and in monkeys.[21]

TABLE 2

Blood sugar

The dogs and the conditions are as shown in figure 1. The blood sugar values are given as mg. per cent.

		Fasting					Fat meal						Growth hormone, fasting						Growth hormone, fed						
Dog no.	Pp* Pi†	6	18	24	42	66	0	4	6	8	12	24	42 0	46 4	48 6	54 12	66 24	90 48	18 0	6 6	18 24	18 48	18 72	18 96	18 120
518																			61			78	74	88	52
521																			43			64	86	113	144
647																			55			130	202	112	234
771		69	72	65	54	53	58	61	54		57	49	72	69	72	63	72	89	58	70	72	90	94	83	102
774		63	57	49	49	54	71	67	65		63	60	69	63	61	62	67	84	55	67	81	92	187	210	258
471		74	84	73	62	54	66	90	88		87	81	62	48	37	131	141		74		90				117
859		82	83	73	65	55	65	55	56		70	73	65	74	77	87	63		76		89				103
860		77	66	67	53	49	53	42	44		66	64	46	83	69	72	83		73		75				94
759													35	73	76	88	87		67		170				303

*Pp:—hours postprandial.
†Pi:—hours after first injection of growth hormone.

DISCUSSION

The physiological role of the nonesterified or unesterified fatty acids (NEFA or UFA) of the blood has been discussed by Dole,[12] Gordon,[13] and others. This fraction is derived mainly from the lipids of the adipose tissue and is transported in combination with albumin. Although it constitutes about 5 per cent of the total fatty acids of the plasma[12] the rapidity of its turnover is so great as to suggest that it may be the active form in which lipid is transported from fat depots to the sites of utilization.[12] The UFA are increased in man by fasting,[13] and this occurred also in our dogs. After a continuous rise during the initial stages of fasting a plateau of considerable height was finally reached. The serum remained clear and the total lipids remained low.

Following a fat meal, the absorbed lipids (mostly triglycerides) were transported from the intestine to sites of storage and utilization in a different fashion—chiefly as chylomicra, as indicated by marked opacity of serum. The nonsignificant rise of UFA after this fat meal is probably due to the very high fat and the low carbohydrate content of the diet. Therefore, the expected fall of UFA observed when carbohydrate is fed to the fasting animal[12] may not be anticipated.

Growth hormone causes an increase in liver lipid, ketonemia, and a lowering of the respiratory quotient in the fasting animal. These changes are indicative of fat mobilization.[14] In this state of enhanced transport of fat from body depots the UFA rose sharply beyond the levels due to fasting alone. While a protein-sparing effect is exerted by growth hormone in the fasting animal,[15,16] the rapidity of the rise in UFA would suggest that the fat mobilization is not subsequent to changes in protein metabolism. It is felt, therefore that the rapid rise in UFA is one of the primary effects of growth hormone.*

Much experimental evidence, summarized by de Bodo and Altszuler,[17] indicates that repeated injections of growth hormone in regularly fed animals enhance the mobilization and utilization of fat and the synthesis of protein. Under these conditions a prediabetic or diabetic state is produced in dogs,[14] and in the present experiments, five of the nine dogs given growth hormone for five days exhibited hyperglycemia and glycosuria (table 2). We also found that the lipids of the plasma were altered considerably when dogs were fed and given growth hormone. The turbidity of the serum which occurred within the first two days of injection is unexplained at present, but must be a part of the general, suddenly increased fat mobilization. The serum phospholipids, total cholesterol, the α-lipoproteins and the β-lipoproteins rose higher than at any other time. The total lipids in the "fasting" serum samples of growth-hormone treated animals were higher than after a fatty meal in the same dogs, yet no turbidity was present after five days of injection. Thus, in this state of enhanced mobilization of lipid, the total lipids and the serum triglycerides rose greatly but remained soluble; apparently, the rise in phospholipids, reflected by rises in the lipopro-

* This view is in agreement with results obtained by F. L. Engel, who used growth hormone in in vitro studies.[22]

teins may explain why the triglycerides did not cause any turbidity and the plasma stayed clear in this experimental condition. The significant increase in UFA is another indication for the enhanced mobilization of lipid from the depots in the prediabetic state induced by growth hormone in regularly fed dogs. It was found that this increase in UFA was accompanied by an increase in α and β lipoprotein and that there was a rough correspondence in the degree of change of these three fractions. The explanation of this relationship is not known at present, and may be coincidental. It is also possible that our observations are related to the results of Lindgren and Gofman[18] who found that 6 per cent by weight of the lipid content of the α lipoproteins of human serum are UFA. According to this view, the UFA (NEFA) are thought to be combined in the serum mainly with albumin, but to a lesser extent (one third) also with the α and β-lipoproteins.[19] Thus it appears that one of the many possible roles of the lipoproteins may be to participate in the transport of UFA during their mobilization from the depots.

SUMMARY

Lipid transport during fat absorption. The chief characteristic of serum lipids during fat absorption is a rise in total lipids. This fat load appeared to be carried chiefly as triglyceride in the form of chylomicra, as indicated by an increase in optical density.

Lipid transport during fat mobilization. In the fasting dog given growth hormone, the mobilization of lipid was manifested by a rapid rise in serum UFA, above that due to fasting alone. The serum remained clear and no characteristic changes occurred in its total lipid, phospholipid or total cholesterol. It is felt that the increase in UFA is one of the primary effects of growth hormone.

Lipid transport in the prediabetic state caused by growth hormone. In fed dogs repeatedly injected with growth hormone, a prediabetic state is produced. In this condition lipid mobilization is greatly enhanced. The total lipids rose to high levels, resulting in lipemia without turbidity in the eighteen-hour "fasting" blood samples after the third day. The phospholipids and cholesterol rose markedly reflected by rises in the α and β lipoprotein fractions. The increases in α and β lipoprotein indicate that these fractions may be involved in the transport of large amounts of serum triglycerides in solubilized form in this prediabetic state. A significant, sustained rise in UFA is part in this state of greatly increased fat mobilization. A portion of these elevated UFA is probably carried in the lipoproteins.

SUMMARIO IN INTERLINGUA

Effectos De Hormon De Crescentia Super Le Transporto De Lipidos In Sanguine

Transporto de lipidos durante le absorption de grassia. Le principal characteristica del lipidos seral durante le absorption de grassia es un augmento de lipido total. Il pare que le carga de grassia es portate principalmente como triglycerido in le forma de chylomicrones. Isto es indicate per un augmento del densitate optic.

Transporto de lipidos durante le mobilisation de grassia. In canes in stato jejun que ha recipite hormon de crescentia, le mobilisation de lipido es manifeste in le augmentation rapide del non-esterificate acidos grasse in le sero. Iste augmentation excede le nivello explicabile per le jejuno sol. Le sero remane clar e nulle alteration characteristic occurreva in su lipido total, in su phospholipido, o in su cholesterol total. Es opinate que le augmento del non-esterificate acidos grasse es un del effectos primari del hormon de crescentia.

Transporto de lipidos in le stato prediabetic que es causate per hormon de crescentia. In canes in stato alimentate que ha recipite repetite injectiones de hormon de crescentia, un stato prediabetic es producite. In iste stato, le mobilisation de lipidos es grandemente promovite. Le lipidos total attinge alte nivellos, con le resultato de lipemia sin turbiditate in le specimens de sanguine "jejun" a dece-octo horas a partir del tertie die. Le phospholipidos e le cholesterol es marcatemente augmentate. Isto es reflectite in augmentos del fractiones alpha e beta de lipoproteina. Iste augmentos indica que le fractiones in question es possibilemente concernite in le transporto de grande quantitates de triglyceridos seral in forma solubilisate que occurre in iste stato prediabetic, viste que un significative e continue augmento del non-esterificate acidos grasse es un characteristica del stato de grandemente augmentate mobilisation de grassia. Un portion del augmentate non-esterificate acidos grasse es probabilemente portate in le lipoproteinas.

ACKNOWLEDGMENTS

The authors appreciate Dr. Irvine H. Page's interest in this study. They are grateful to Dr. Charles H. Best for his help and wish to acknowledge the excellent, technical work of Mrs. V. Lazdins.

The investigation was aided by grants from the National Research Council of Canada, the Nutrition Foundation, Inc., and the Life Insurance Medical Research Fund, New York. The lipoprotein part of these studies was financed in part by a grant from the National Heart Institute, United States Public Health Service, Bethesda, Maryland.

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The conversion of certain pentoses to glucose was suggested by the finding that the infusion of 5 to 20 gm. of D-xylose or L-arabinose resulted in a 10 to 30 per cent increase in blood glucose levels. On infusion none of the pentoses affected either the blood lactate or pyruvate concentrations, but all caused a significant decrease in the level of serum inorganic phosphate, this being most marked in the case of D-xylose. In order to obtain information on the fate of the relatively large fraction of D-xylose which did not appear in the urine, two experiments were performed with D-xylose-1-C^{14} in which the disposition of the isotope was determined. Virtually all of the C^{14} recovered from the urine was found in the xylose band on chromatography, less than 0.1 per cent appearing as xylitol or xylonolactone which are two possible metabolites. The expired CO_2 was maximally labeled forty-five minutes after the infusion ended, although the first CO_2 sample collected five minutes after D-xylose-1-C^{14} was injected was already appreciably labeled. C^{14} was present in expired CO_2 in detectable amounts for six hours and the total $C^{14}O_2$ expired represented 13.5 per cent of the administered label. The similarity of the CO_2 labeling pattern after C^{14} D-xylose and C^{14} D-glucose suggests that these sugars probably share common intermediary pools which have a rapid turnover. The fate of the residual 30 per cent of the administered label, as well as of the lower four carbons of the xylose whose first carbon appeared as CO_2, remains obscure.

From *Nutrition Reviews,* Vol. 16, No. 7, p. 202, July 1958.