Mobilization of Free Fatty Acids from Adipose Tissue from Normal and Diabetic Subjects

Influence of Glucose and Insulin

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Free fatty acids (FFA) constitute an important form in which fat leaves the storage depots for utilization elsewhere in the body.[1] Studies in vivo have shown that increasing quantities are mobilized, so that their concentration rises in the circulating blood, whenever there is an increased oxidation of fat in the body, as occurs during starvation or in diabetes mellitus.[2-5] On the other hand, the rate of mobilization and the level of FFA in the blood falls when carbohydrate is more readily available, such as occurs following a meal or an infusion of glucose,[2-4] or when the diabetic state is controlled with insulin.[5-6] In diabetic ketoacidosis this fall may be the first demonstrable event following treatment and precede any reduction in the blood sugar level.[6] These studies suggested that the state of carbohydrate metabolism in the body might be an important factor controlling how much FFA is released from the depots for utilization by the peripheral tissues.

In vitro studies have confirmed many of the inferences drawn in life. Adipose tissue from starved rats releases more FFA than does tissue from fed animals,[7-9] and the release is greater from adipose tissue of alloxan diabetic than from normal animals,[10] whilst the addition of glucose and insulin in vitro decreases the quantity of FFA liberated from normal tissue.[8,9]

The early concept that adipose tissue is merely a passive and inert structure for the storage of fat is no longer tenable,[11] and it has been shown to utilize carbohydrate actively and synthesize large quantities of fatty acids.[12-14] Variations occur in its activity in relation to the nutritional status of the animal[11] and it is particularly sensitive to the action of insulin.[14-16] Local alterations in the quantity of glucose utilized by the adipose tissue cells are likely, therefore, to play an important role in regulating the rate of mobilization of FFA from the depots. In the present study, the release of FFA and their concentration within the epididymal fat pads of normal and alloxan diabetic rats have been measured and the sensitivity of the tissue to glucose and insulin determined.

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METHODS

Epididymal fat tissue was obtained from normal and alloxan diabetic rats. The animals were anesthetized with intraperitoneal Nembutal (0.05 - 0.1 mg. per gram body weight), and immediately after removal each pad was placed in Krebs-Ringer bicarbonate buffer (warmed to room temperature). The pads were divided by a longitudinal incision into two equal halves, so that each half contained corresponding amounts of both the thickened proximal part and the thinner distal tissue. Each animal yielded four pieces of tissue which were found to have comparable activity (see Results). The half pads were lightly blotted, weighed and incubated in 4 ml. volumes of Krebs-Ringer bicarbonate buffer containing 5 per cent serum albumin (w/v), at 37° C. in an atmosphere of 95 per cent O_2 — 5 per cent CO_2.

A 1 ml. sample of the medium was withdrawn after ten minutes to allow for equilibration between the tissue and the medium, and duplicate 1 ml. samples were removed at the end of a two-hour incubation and the FFA contents determined by the method of Dole.[3] The difference between the initial and final concentrations measured the net quantity of FFA released into the medium. When the content of FFA within the fat pads was to be measured, the pads were washed in several changes of ice-cold Krebs-Ringer bicarbonate buffer and immediately homogenized in the same extraction mixture as is used in the Dole method, and the quantity of FFA then determined in the usual way. FFA have been expressed in terms of their equivalence of palmitic acid in μmoles per gram of wet tissue. The mean value in each series of experiments has been taken and its standard error calculated.

Diabetes mellitus was induced by an injection of alloxan (15 mg./100 gm. body weight), and the animals were kept after treatment until they showed glycosuria and ketonuria and the blood sugar rose to values

greater than 270 mg./100 ml.

Glucose was measured as total reducing substance by the method of Nelson.[17] The total reducing capacity of concentrated "stock" solutions of serum albumin was measured and it was calculated that the apparent concentration of glucose from this source in the final incubating medium was never greater than 0.275 mM., an amount that does not have any demonstrable effect on the release of FFA.[18]

RESULTS

RELEASE OF FREE FATTY ACIDS

Comparison of halved pads from individual animals. The four half-pads (obtained by a longitudinal division of each pad) from any individual animal behaved in a very similar manner, although in any comparable group of rats the value varied between different animals (table 1). The standard error of the activity of the four half-pads in any single animal in this, and in other series of experiments, did not exceed ± 0.09. Consequently, one half-pad could act as a control for the others, whose behavior could be determined under varying experimental conditions.

Comparison of fat pads from normal and diabetic animals:

Nutritional status. The nutritional status of the animal had a marked effect on the subsequent release of FFA from the adipose tissue (figure 1). If normal rats were allowed free access to food before killing, the release was only 0.27 ± 0.11 μmoles per gram of tissue per hour, but starvation for 24, 48 and 72 hr. respectively, resulted in a progressive increase in the amount to a value of 2.73 ± 0.17 μmoles per gram per hour. It was not found possible to starve diabetic rats for more than four to eight hours, because of the high mortality induced thereby, but even so, the release of FFA was greater than that from the tissue of normal animals starved for seventy-two hours. The amount varied to some extent in proportion to the severity of the diabetic state, as assessed by the blood sugar level at the time of killing, but there was no close relationship (figure 2).

TABLE 1

Comparison of the release of FFA from halved epididymal fat pads of individual animals (FFA expressed as μmoles/gm. of tissue per hour of incubation)

Animal	One fat pad		Other fat pad		Mean value	Standard error
	Half pad	Half pad	Half pad	Half pad		
1	1.53	1.76	1.54	1.66	1.62	± 0.07
2	1.93	2.15	1.92	2.06	2.02	± 0.06
3	1.73	1.90	2.01	2.19	1.96	± 0.08
4	2.12	1.82	2.24	1.90	2.02	± 0.09
5	1.68	1.47	1.43	1.26	1.46	± 0.08
6	2.25	2.12	2.23	2.08	2.17	± 0.05

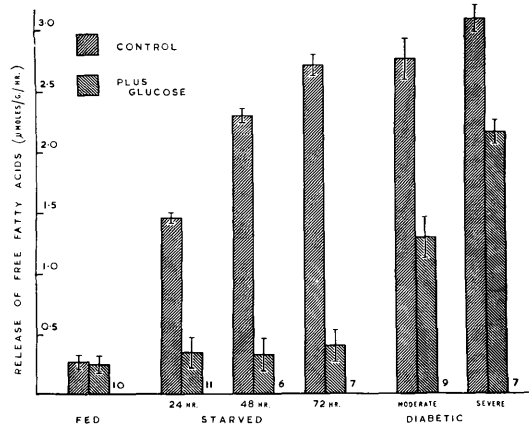

FIG. 1. Comparison of the release of FFA from epididymal fat tissue of normal and alloxan diabetic animals. Normal animals were fed or starved for twenty-four, forty-eight, and seventy-two hours, respectively; diabetic animals were allowed free access to food. Blood sugars at the time of killing, moderate diabetes <450 mg./100 ml., severe diabetes >450 mg./100 ml. In each group, the left column represents the control release and the right column the release from paired tissue when 5.5 mM. glucose added. Mean values are given with their standard errors (number of experiments is stated at the base of the columns).

Effect of glucose. In each experiment the effect of added glucose was compared directly with control tissue from the same animal. Glucose, in a physiological concentration of 5.5 mM., had no effect on the release of FFA from adipose tissue of fed normal rats, but caused a marked reduction when added to tissue from starved animals (figure 1). The resultant values were similar, irrespective of the duration of starvation, and were

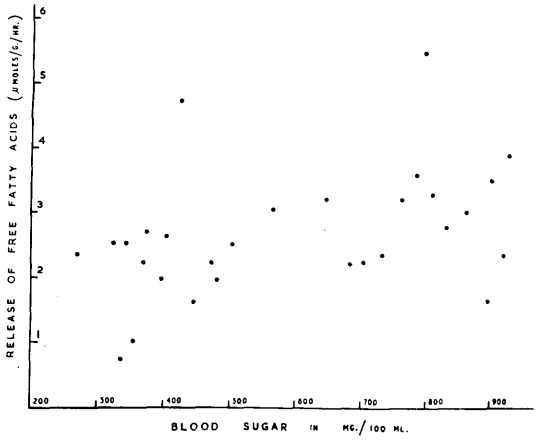

FIG. 2. Release of FFA from epididymal fat pads of individual alloxan diabetic rats. FFA are expressed as μ moles per gram of tissue per hour of incubation. Severity of diabetes assessed on the basis of the blood sugar level at the time of killing.

comparable to the control release from fed tissue. Glucose alone similarly decreased the release of FFA from the fat pads of diabetic animals, but its effect was much less marked, and its efficacy declined as the severity of the diabetes worsened.

The inhibitory action of glucose decreased progressively as its concentration was reduced (table 2), but adipose tissue from starved animals showed a great sensitivity even to small quantities. The minimum effective concentration of glucose in this case was 0.67 mM. and at this level the release of FFA was reduced to 36 per cent of the control. Comparable concentrations of glucose were much less effective on diabetic tissue, and no significant response could be detected with concentrations less than 1.3 mM.

Effect of glucose and insulin. In each animal, one half-pad acted as the control, whilst to the others were added glucose and insulin separately and combined (table 3). Both in the case of tissue from starved normal and also from diabetic animals, the additional presence of insulin did not cause any further reduction in the release of FFA than occurred with glucose alone. Insulin was without effect on the release of FFA, even though it increased the uptake of glucose twofold in both groups of animals. Moreover, when insulin was added alone the value was no different from the control.

CONTENT OF FFA WITHIN THE FAT PADS

Comparison of halved pads from individual animals. The quantity of FFA within the half-pads was measured immediately after killing the animals and following a control incubation. In each animal, the amount of FFA within the half-pads was similar under comparable conditions (table 4). This suggested that little autolysis of storage triglyceride occurred during the extraction procedure, so that the values measured approximated closely to the actual quantity present immediately before extraction. Moreover, in any single animal the amount in one pad could be used as a measure of that

TABLE 2

Effect of decreasing concentrations of glucose on the release of FFA from halved fat pads of forty-eight-hour starved normal and alloxan diabetic animals. The diabetic rats were not starved, and their blood sugar at the time of killing ranged from 270 to 500 mg./100 ml. FFA are expressed as μmoles per gram of tissue per hour. The mean values for each series of experiments are given together with the standard errors.

Concentration of glucose (mM)	Forty-eight-hour starved normal animals			Diabetic animals		
	Number of experiments	Control	+ added glucose	Number of experiments	Control	+ added glucose
5.5	8	2.24 ± 0.19	0.27 ± 0.09	6	2.46 ± 0.11	1.27 ± 0.19
2.75	6	2.31 ± 0.16	0.56 ± 0.17	5	2.81 ± 0.21	1.55 ± 0.18
1.325	5	2.11 ± 0.09	0.74 ± 0.29	4	2.50 ± 0.14	1.58 ± 0.21
0.667	4	1.40 ± 0.20	0.50 ± 0.32	5	2.69 ± 0.22	2.81 ± 0.23
0.334	5	1.38 ± 0.24	1.28 ± 0.33		—	—

TABLE 3

Effect of glucose and insulin on the release of FFA from adipose tissue of forty-eight-hour starved normal and alloxan diabetic animals. In each animal, one half pad acted as the control, while to the others were added glucose or insulin separately or combined. The mean release of FFA in each group is expressed in μmoles per gram of tissue per hour and the S.E. calculated (the number of experiments is stated in parentheses). The concentration of glucose used was 5.5 mM. and that of insulin was 0.01 to 0.1 unit/ml. of medium.

Description	Release of FFA			
	Control	Glucose	Glucose + insulin	Insulin
Forty-eight-hour starved normal animals (9)	1.68 ± 0.17	0.28 ± 0.05 *(1.18 ± 0.12)	0.26 ± 0.09 *(2.52 ± 0.19)	1.79 ± 0.20
Diabetic animals				
(a) Moderate (blood sugar on sacrifice <450 mg./100 ml.) (6)	2.38 ± 0.08	0.88 ± 0.38 *(0.73 ± 0.31)	1.01 ± 0.46 *(1.57 ± 0.27)	2.53 ± 0.22
(b) Severe (blood sugar on sacrifice >450 mg./100 ml.) (5)	3.20 ± 0.28	2.12 ± 0.25 *(0.52 ± 0.21)	2.16 ± 0.29 *(1.31 ± 0.39)	3.41 ± 0.32

*Mean uptake of glucose (with S.E.) expressed as milligram per gram of tissue.

in the others. In subsequent studies, the content of FFA was measured in one half-pad immediately after killing and in the others after a two-hour incubation, whilst the simultaneous release into the medium was also measured. The total quantity of FFA present at the end of an incubation would comprise those remaining within the pad together with the amount passing out into the medium, and a close measure of the quantity present at the start of the incubation was given by the content of FFA in the half-pad that was determined immediately after killing the animal. Consequently, an estimate could be made of any net change in the total quantity of FFA during the incubation.

TABLE 4

Comparison of the content of FFA within the halved fat pads of individual animals (values expressed in μmoles per gram of tissue)

Animal	Description	Content of FFA within the half pads			
		Left pad		Right pad	
		Half	Half	Half	Half
1	On death	3.32	2.99	3.05	2.88
2	On death	1.65	1.27	1.49	1.34
3	On death	2.37	2.25	2.19	2.07
4	On death	2.12		1.93	
	After incubation		2.34		2.01
5	On death	2.01		1.92	
	After incubation		2.16		2.20
6	After incubation	2.30	2.20	2.10	1.90

Comparison of normal and diabetic tissue. The initial content of FFA within the pads at the time of killing was lowest in the fed animals; it increased with starvation and was three times greater in the diabetic tissue (table 5). During the subsequent control incubation, the total quantity of FFA present increased by less than 20 per cent in the case of fed tissue, whereas it approximately doubled in the experiments with forty-eight-hour starved and diabetic tissue.

Effect of glucose and insulin. Both in the case of tissue from forty-eight-hour starved normal and also from diabetic animals, the addition of glucose (or glucose and insulin) resulted in the final quantity of FFA within the pads being less than that remaining in the control tissue (table 5), and comparison with the estimated amount present at the start, showed that the total quantity of FFA actually decreased during the incubation. There was no significant difference between the effects of glucose alone and those of combined glucose and insulin on either the concentration of FFA in the pads or the quantity released into the medium. Insulin was equally without effect both on starved normal and on diabetic tissue, although it doubled the uptake of glucose by both tissues. Furthermore, insulin by itself had no effect on the quantity of FFA present.

In all the experiments performed, there was a close relation between the quantity of FFA within the pads and the magnitude of their net release into the medium during the incubation; the higher their concentration within the tissue the greater the quantity escaping into the medium (figure 3).

DISCUSSION

Previous studies have shown that the release of FFA from epididymal fat pads of fed normal animals was small, but progressively increased as starvation continued,[8,9] and this has been confirmed (figure 1). However, in the case of tissue from diabetic animals high values were seen even though the animals could not be starved before killing. The addition of glucose had little effect in further decreasing the already small release of FFA from fed tissue, but caused a considerable reduction in that from starved animals. The extent of this reduction declined progressively as the amount of glucose added was decreased, but the tissue remained extremely sensitive even to small quantities. The lowest effective concentration was 0.67 mM. and this level corresponds closely with the smallest measurable quantity of glucose-U-C-14 that can be metabolized in vitro.[19,20] Glucose had a much smaller inhibitory effect on the release of FFA from diabetic than from starved normal tissue and this difference was associated with a lower uptake of glucose by the former tissue. These findings suggested that the rate of cellular utilization of glucose in vitro exerted an important controlling influence on the mobilization of FFA from the adipose tissue.

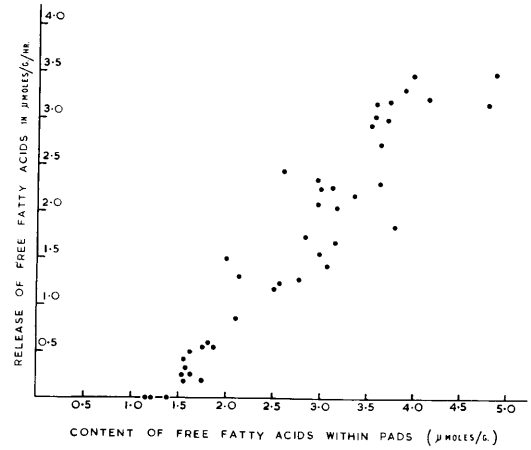

FIG. 3. Relationship between concentration of FFA within epididymal fat pads and the quantity released into the medium.

Estimation of the total quantity of FFA present in

TABLE 5

Change in the total quantity of FFA present during a two-hour incubation. The number of experiments is stated in parentheses in each case. The mean content of FFA is expressed in μmoles per gram of tissue and the mean release in μmoles per gram per hour, and the S.E. calculated. Concentration of glucose used was 5.5 mM. and insulin 0.01 to 0.1 unit/ml.

Description	Half pad Content when killed **a**	Half pad Content after incubation **b**	Release into the medium **c**	Total in the system **d = b + 2c**	Calculated change **d − a**
Normal tissue					
Fed (6)	1.79 ± 0.24	1.58 ± 0.28	0.26 ± 0.08	2.10 ± 0.17	+ 0.31 ± 0.12
Forty-eight-hour starved (5)	3.45 ± 0.11	2.55 ± 0.10	2.45 ± 0.22	7.45 ± 0.77	+ 4.00 ± 0.44
Diabetic tissue (6)	5.15 ± 0.56	3.59 ± 0.44	3.19 ± 0.24	9.97 ± 0.76	+ 4.82 ± 0.59
Forty-eight-hour starved—normal tissue		Control incubation			
(8)	3.67 ± 0.20	3.00 ± 0.52	2.26 ± 0.25	7.52 ± 0.92	+ 3.85 ± 0.64
		Glucose			
		1.54 ± 0.18	0.25 ± 0.06	2.04 ± 0.27	— 1.63 ± 0.31
		Glucose and insulin			
		1.76 ± 0.37	0.16 ± 0.26	2.08 ± 0.40	— 1.59 ± 0.47
		Insulin			
		3.82 ± 0.57	1.76 ± 0.32	7.34 ± 0.53	+ 3.67 ± 0.51
Diabetic tissue (6)		Control incubation			
	5.69 ± 0.68	4.86 ± 0.75	3.16 ± 0.34	11.18 ± 0.94	+ 5.49 ± 0.87
		Glucose			
		1.97 ± 0.49	1.57 ± 0.15	5.11 ± 0.88	— 0.58 ± 0.26
		Glucose and insulin			
		2.10 ± 0.21	1.42 ± 0.17	4.94 ± 0.73	— 0.75 ± 0.30
		Insulin			
		4.94 ± 0.88	3.45 ± 0.52	11.84 ± 0.93	+ 6.15 ± 0.89

the system clearly demonstrated that, instead of the anticipated rise during incubation, the addition of glucose resulted in an actual decrease. In every case, the decreased release of FFA was associated with a fall in their level within the pads, and comparison of the values (figure 3) confirmed that the magnitude of the net release closely paralleled their concentration within the tissue.[7,20] The inhibitory action of glucose on the mobilization of FFA would seem, therefore, to be largely a consequence of its effect in decreasing their concentration within the cells.

It has been shown that glucose increases the uptake of palmitate-1-C-14 by adipose tissue in vitro and stimulates its incorporation into storage triglyceride.[20–22] Moreover, it also acts as a source for the synthesis of new fatty acids[12–14] and decreases their rate of oxidative breakdown.[20] Both the latter two actions might be expected to increase the concentration of FFA in the tissue, whereas in fact their level was reduced. Consequently, the predominant effect of glucose appeared to be one of stimulating the rate of esterification, thereby reducing the concentration of FFA within the cells.

Adipose tissue is extremely sensitive to the action of insulin,[14–16] and in the present experiments it increased the uptake of glucose twofold. However, despite this, its additional presence did not cause significant further reduction in the release of FFA nor any further decrease in their concentration within the pads, beyond that of glucose alone. This lack of effect of insulin was seen with tissue both from normal and also from diabetic animals, and suggested that although it increased the translocation of glucose into the cells, insulin had little effect in stimulating the rate of esterification of FFA. Such a conclusion is in accord with the similar findings that glucose alone increases the uptake of palmitate-1-C-14 by adipose tissue and its incorporation into triglyceride, whereas additional insulin has little further effect.[20]

Glucose-carbon is readily incorporated into the glycerol part of the triglyceride molecule,[19,23] and studies suggest that glucose stimulates the esterification of FFA by virtue of its conversion into phosphorylated glycerol,[21,24,25] most probably L-α-glycerophosphate, which appears to be an essential intermediary for the maximal synthesis of triglyceride in adipose tissue.[26] The fat depots have only a limited capacity to phosphorylate free glycerol and metabolize it,[22,24,27] so that free glycerol has little effect in stimulating the esterification of FFA and thereby altering their rate of uptake[27] or release from the tissue.[18] A continuous interconversion of triglyceride and FFA takes place within the depots,[20,24] but lipolysis produces free glycerol,[22,28] which is therefore largely unavailable for re-esterification purposes. Consequently, adipose tissue would seem to be dependent on a supply of glucose, for conversion to dihydroxyacetone phosphate and subsequent reduction to glycerophosphate, to enable the FFA to be esterified. In other tissues, such as muscle and yeast, although no data are available for adipose tissue, the equilibrium mixture of the triose phosphates produced during glycolysis, consists of 96 per cent dihydroxyacetone phosphate and 4 per cent D-glyceraldehyde-3-phosphate.[29] If the equilibrium favors dihydroxyacetone phosphate in a similar manner in adipose tissue, it could explain why so much glucose-carbon can be converted into presumptive glycerophosphate and so incorporated into triglyceride in the depot cells. It has been calculated that, even in the absence of insulin, glucose yields a large relative excess of presumptive glycerophosphate,[23] so that esterification of FFA may, therefore, be stimulated almost maximally by glucose alone. If this be so, little further stimulation will be possible with insulin, even though its presence increases the uptake of glucose considerably. This might explain why additional insulin had little greater effect than did glucose alone in increasing the rate of esterification in this, and in other studies.[20]

New synthesis of fatty acids and their continuous production by lipolysis of storage triglyceride[20,24] will lead to an accumulation of FFA unless esterification proceeds normally. Variations in the rate of esterification may, therefore, be important in determining their concentration within the cells and thereby in controlling their rate of mobilization from the depots. Moreover, a dependence of the tissue on carbohydrate metabolism for adequate esterification to occur, and variations in the availability of glucose for utilization by the depot cells, can explain the differences in behavior observed between the tissue of fed and starved animals. The initial content of FFA in the pads of fed animals was low at the time of killing, and during the subsequent incubation there was little change in the total quantity present. The addition of glucose caused little if any reduction in the release of FFA, suggesting that in fed tissue there was adequate re-esterification of the FFA produced by lipolysis. During progressive starvation less carbohydrate and its intermediates were available for utilization by the depots in life and this was associated with a rise in the level of FFA in the pads at the time of killing, and a twofold increase in the total quantity present during the subsequent control incubation. Irrespective of the duration of starvation, glucose decreased the release of FFA to a final value similar to the control release from the fed tissue. Moreover, glucose reduced the total quantity of FFA to a value similar to that in the fed tissue at the time of killing. These observations suggested that there was an impaired rate of esterification in the tissue of twenty-four- to seventy-two-hour starved animals due to a lack of available glucose. This impairment of esterification could account for most, if not all, of the increased mobilization of FFA and seemed to be the major factor controlling the rate during starvation.

Similarly, there was a high initial content of FFA in the pads of diabetic animals at the time of killing, whilst glucose decreased the value of their subsequent release in vitro and reduced the quantity present to less than that at the time of killing. Again, there appeared to be an impaired rate of esterification, but the effect of glucose was much less than that seen with starved normal tissue and the release of FFA was only reduced to 50 to 60 per cent of the control. Part of this difference in behavior may have been due to the lower uptake of glucose, but factors other than simply an impaired rate of esterification may also have been responsible for the increased release of FFA. This is suggested by the findings that there is only a poor correlation between the release in vitro and the severity of the diabetic state as judged by the blood sugar level[10] and this has been confirmed, whilst a similar lack of correlation has also been found in vivo.[30] An increased rate of lipolysis may occur in diabetes and be induced, for example, by an increased secretion of the lipolytic hormones epinephrine or growth hormone. Evidence in support of this was the observation that adrenergic blockade with dibenzaline caused a very marked reduction in the release of FFA from diabetic tissue.[25] Moreover, if lipolysis is already increased the extent of any possible further increase may be limited, and this would explain the observation (unpublished data) that corresponding concentrations of either epinephrine or

growth hormone produce a much smaller relative increase in the release of FFA from diabetic than they do from normal tissue.

SUMMARY

The quantity of free fatty acids (FFA) released from epididymal fat pads in vitro and their concentration within the tissue were determined. These quantities paralleled one another and were low in the case of tissue from fed animals, but were both increased with tissue from twenty-four- to seventy-two-hour starved normal and alloxan diabetic animals. The addition of glucose had little effect on their levels in the former but decreased them very considerably in the case of starved rats and to a lesser extent in the diabetic animals. The additional presence of insulin increased the uptake of glucose both by normal and by diabetic tissue, but in neither case did it cause any further reduction in the quantity of FFA.

The findings suggested that the rate of cellular utilization of glucose in vitro had an important controlling effect on the release of FFA, and that the extent of the inhibitory action of glucose on the release was largely a manifestation of its activity in decreasing the concentration of FFA within the cells. The predominant effect of glucose was to increase the rate of esterification of FFA, but insulin appeared to have little effect on this. Inadequate esterification could account for the increased mobilization of FFA during starvation and seemed to be the major factor controlling it, whereas in diabetic animals it was likely that in addition an increased rate of lipolysis was also partly responsible.

SUMMARIO IN INTERLINGUA

Le Influentia de Glucosa e de Insulina Super le Mobilisation de Libere Acidos Grasse ab Normal e Diabetic Tissu Adipose

Esseva determinate le quantitate de libere acidos grasse que es liberate per cossinos de grassia epididymal in vitro e etiam le concentration de illos intra le tissu. Iste quantitates esseva in parallela le unes con le alteres, e illos esseva basse in le caso de tissu ab alimentate animales, sed illos esseva augmentate in tissus ab normal rattos post inter vinti-quatro e septanta-duo horas de jejuno e etiam ab rattos diabetic per alloxano. Le addition de glucosa non alterava significativemente le nivellos in le prime del duo casos, sed illo reduceva los considerabilemente in le caso del jejunate rattos e, minus marcatemente, in animales diabetic. Le presentia additional de insulina augmentava le acceptation de glucosa per tissue normal e etiam per tissu diabetic, sed illo causava—ni in le un ni in le altere caso—ulle reduction additional in le libere acido grasse.

Le constatationes suggere que le intensitate del utilisation cellular de glucosa in vitro habeva un significative effecto regulatori in le liberation de libere acido grasse e que le grado del effecto inhibitori de glucosa super ille liberation es predominantemente un manifestation de su activitate reductori in le concentration de libere acido grasso intra le cellulas. Esseva trovate que le predominante effecto de glucosa consisteva in accelerar le esterification de libere acido grasse, sed il pareva que insulina ha pauc effecto in isto. Inadequate esterification explica possibilemente le augmentate liberation de libere acido grasse durante periodos de jejunation e pareva esser le major factor in su regulation, durante que in animales diabetic il pareva probabile que in plus un accelerate lipolyse esseva etiam responsabile in parte.

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Dietary-induced Atherosclerosis and Thrombosis in the Dog

A form of arteriosclerosis can be produced readily in rabbits and chickens by simply supplementing the diet with cholesterol. However, thrombosis rarely occurs. Arterial thrombosis can be produced in rats by more complex diets but the amount of associated arteriosclerosis is usually insignificant. In man we frequently find both thrombosis and arteriosclerosis and it would be useful if we could develop an experimental model that had both. With this object in mind, N. R. DiLuzio and R. M. O'Neal (Exp. Molec. Path. *1*:122, 1962) and P. M. Hartroft, M. Suzuki, and O'Neal (Ibid. *1*:133, 1962) have fed dogs diets previously used to produce thrombosis in rats ("infarct-producing" diets). One of their objectives was to see if the diets would also produce thrombi in dogs and another to determine whether arteriosclerotic lesions could be produced that were more severe than those seen previously in the rat.

The experiments were carried out independently in two different laboratories with fifteen dogs in one and four in the other. The experimental diets contained thiouracil, bile salts, cholesterol, and butter plus adequate casein, sucrose, minerals, and vitamins.

In the experiments of DiLuzio and O'Neal, with ten dogs receiving the experimental diet and five controls fed a stock diet for three to nineteen weeks, no thrombi or infarcts were found. However, gross aortic lesions were demonstrated in all of the eight dogs receiving the experimental diet who survived for eight weeks or longer and chemical analyses of their aortas showed greater amounts of lipid, especially cholesterol ester, in most than were found in the controls. Most were extremely hyperlipemic, with total lipids in plasma as high as 11 gm. per 100 ml.

In the experiments of Hartroft, Suzuki, and O'Neal the experimental diet was fed for as long as one year. One dog developed thrombosis of the superior mesenteric artery and died of infarction of a portion of the intestinal tract. Extensive arterial lesions containing lipid were found in this dog.

In both studies the investigators concluded that they had demonstrated the suitability of the dog for the dietary production of atherosclerotic lesions of the "cholesterol type." The rapidity with which such lesions can be produced is stressed by DiLuzio and O'Neal and the fact that thrombosis may occur as a complication by Hartroft, Suzuki, and O'Neal.

The authors quote the results of their earlier work to the effect that the low fat diet was associated with a reduction in total fatty acid concentration in the serum, and that this reduction was not influenced by caloric intake (Wiese, Hansen, and M. A. Baughan, J. Nutrition *63*:523, 1957). In the present article, serum concentrations of various individual fatty acids are given,

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