RES MEDICA
Journal of the Royal Medical Society

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B.Sc., M.B., Ch.B. , Senior President 1963-64.

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ISSN: 2051-7580 (Online) ISSN: 0482-3206 (Print)
Res Medica is published by the Royal Medical Society, 5/5 Bristo Square, Edinburgh, EH8 9AL

Res Medica, Autumn 1964, 4(3): 28-33
doi: 10.2218/resmedica.v4i3.431

"OBSERVATIONS ON LIPID METABOLISM"

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Prior to 1936, it was the custom to regard adipose tissue of an accumulation of inert lipid material, possessing little or no metabolic activity, but, in that year, this viewpoint was challenged by the work of Schoenheimer and Rittenberg. On the basis of measurements of the rate of disappearance of labelled fatty acids from the body of the mouse, these workers concluded that the half-life of the total fatty acids in this animal, under the conditions of the experiment, was of the order of three days. Since the depots constitute by far the greatest part of the body fats, it was assumed that the turnover rate observed was that characteristic of the depot fat. The acceptance of these results necessitated the immediate rejection of the long cherished idea that adipose tissue represents an inert lipid store, capable of change only during periods of fasting or of excess ingestion of food.

This "about face" in belief naturally stimulated many workers and confirmatory results appeared rapidly in the literature. Thus, Shapiro and Wertheimer evaluated the oxygen uptake of adipose tissue *in vitro* and took the elementary precaution, or so it appears now, of expressing their results in terms of the fat-free weight of the tissue in this way demonstrating that "depot fat" (so-called) ranked amongst the most active tissues in the body when judged by this criterion. In 1942, Tuerkischer and Wertheimer reported that all dietary regimens which enhance fat formation also give rise to the deposition of glycogen within the adipose tissue cells. Furthermore, these authors pointed out that this accumulation of polysaccharide is associated with high respiratory quotients, often exceeding unity, indicating that active fat synthesis is occurring. More recently, isotopic evidence of fat synthesis in adipose tissue *in vivo* has been obtained by Fararger and Gerlach who found that the fatty acids of rat mesenteric lipid have higher specific activities than those of either liver or blood shortly after the injection of radioactive acetate or glucose.

Isolated enzymes of adipose tissue have, to date, been studied relatively little but it may be assumed, from the fact that the tissue is capable of performing such reaction sequences as those involved in respiration and fat and glycogen syntheses that many enzyme systems do occur in this tissue. There is nothing to be gained by further discussion of these systems here—suffice it to say that the main point established by the endeavours to demonstrate enzymic activities in adipose tissue is its evident marked specialisation in terms of lipid metabolism. While most of those enzymes sought can be detected in this tissue, those concerned with fat metabolism show an activity equal to or exceeding that of their counterparts in the most active tissues of the body.

For many years reports appeared sporadically in the literature of small amounts of non-esterified fatty acid (**Nefa**) which can be detected in plasma, but until the early 1950's this lipid fraction was widely regarded as an artefact of isolation. At this time, however, Gordon pointed out that certain observed "anomalies" in the electrophoretic mobility of plasma proteins may be reproduced *in vitro* by the addition of sodium oleate to plasma prior to the application of the separatory procedure. This

provided the first clue leading to the suggestion that **Nefa** might be a physiological component of the circulating lipids, but it was not until a year had elapsed that Gordon and Cherkes ascribed to this fraction an important role in the transport of fats from the depots to the tissues for oxidation. This contention is supported by several lines of evidence. First, it has been shown that **Nefa** injected into various experimental animals has a circulating half-life of the order of only two minutes, indicating its rapid removal from the blood. Second, estimates made of the arterio-venous differences in plasma **Nefa** levels across various organs have indicated that, for example, the myocardium of the fasted animal is capable of removing 0.3 MEquivalents of **Nefa** from each litre of the perfusing blood. In order to assess the physiological significance of this process, the authors performed concurrent measurements of oxygen uptake and, on the basis of the assumption that the average molecular weight of the fatty acid taken up is 275 (i.e. a mixture of fatty acids quantitatively distributed about the hypothetical C17 compound), they arrived at the conclusion that the quantity of **Nefa** taken up by the heart if sufficient to provide the bulk of its energy requirement under the conditions of the experiment.

The next problem requiring explanation was the mechanism by which the constancy of the arterial **Nefa** level is maintained, a problem to which we shall return later. At this moment, all that need be said is that all the tissues studied, including liver, appeared, on the basis of arterio-venous difference studies, to be active in the extraction of **Nefa** from the circulating fluid. The quest, therefore, was for a source of **Nefa** and the investigators turned to the adipose depots. They were not disappointed for it was found that samples of blood from the long saphenous vein, which may be regarded as draining the adipose tissue of the lower limb almost exclusively, showed large negative arteriovenous differences indicative of **Nefa** release.

Thus the pattern has emerged in which **Nefa** represents an important, readily available source of oxidizable material whose concentration in the blood is the resultant of its rate of removal by the tissues and the rate of its liberation from the fat depots.

We may now turn our attention to a consideration of the concept of "caloric homeostasis". As was noted earlier, the concentration of **Nefa** in the plasma is maintained at a relatively constant level, between the limits 0·5—1 mEq per litre, despite the high rate of removal by the tissues which is observed in the post absorptive state. This implies the operation of some fairly sensitive control mechanism capable of relating the rate of liberation of **Nefa** to the somatic requirement. If we return for a moment to the work of Gordon and Cherkes in which the rate of uptake of Nefa by the myocardium was estimated, we find that their results apply only to the fasting state. In fact, Gordon proceeded in 1957 to repeat the experiments and obtained sequential blood samples for the measurement of arterio-venous **Nefa** differences before, and for some time after, the administration of 100 g. of glucose together with 0.1 unit of insulin per Kg. of body weight to human subjects. In this way he confirmed the previously obtained results and, in addition, showed that the ready availability of glucose, which may be regarded as a preferred metabolite, abolishes not only the uptake of **Nefa** by the myocardium but also its liberation by the adipose tissue. This work provides an extremely elegant example of the close correlation existing between those processes leading to the removal of fatty acids from the circulating fluids and those leading to their mobilization from the fat stores. In general, it may be said that all of those factors which increase the utilization of glucose, decrease the outflow of **Nefa** from the lipid depots. An ingenious explanation of this phenomenon will be quoted elsewhere in this dissertation but, at present, it will suffice to remark that the mobilization of fat shows marked dependence on the state of nutrition, and that pre-eminent in this regard is the status of carbohydrate metabolism.

We have seen, then, that adipose tissue appears to exert its influence on lipid metabolism by adjusting the availability of **Nefa** in accordance with somatic requirements for an oxidisable substrate. This being accepted the central issue quite clearly becomes that of the elucidation of the nature of the mechanism by which the adjustment is effected under physiological conditions. It would be logical to suppose that the process is subject to humoral and to nervous control and indeed, evidence that such is the case has been obtained by many investigators.

The first piece of evidence regarding the role of nervous activity was provided in 1922 by Goering who pointed out that excessive nerve stimulation provokes fat loss from the adipose tissue situated within the distribution of the affected nerve, whereas paralysis or nerve

section results in a marked deposition of fat in the depots, the magnitude of which may be partially or totally masked by the associated atrophy of the somatic musculature. Then, in 1947, Clement reported that unilateral denervation of various fat bodies in the rat caused a diminution in the rate of depletion of triglyceride from the denervated side during fasting. Such observations suggest that the nervous system may exercise a tonic effect upon **Nefa** liberation from fat depots. This concept has found confirmation in the work of Havel and Gotofen (1960) who investigated the role of the sympathetic nervous system in the metabolism of free fatty acids and observed that administration of hexamethonium, a ganglion blocking agent, causes a reduction in plasma **Nefa** levels in the fasting dog. From observations such as these, it has been concluded that the sympathetic nervous system exercises a tonic influence on the adipose tissue which may provide for a continuous release of **Nefa** at a level which can be modified by insulin and other humoral agents. In addition to this, variation in the intensity of sympathetic activity itself might be reasonably expected to exert a direct effect on lipid mobilization.

Many humoral agents have been implicated in the regulation of fatty acid exchange in adipose tissue. We may begin by considering epinephrine. Administration of this hormone to fasting humans has been found consistently to give rise to very rapid and striking increases in the plasma **Nefa** content which can be attributed to an increased rate of mobilization from the fat depots (as shown by arterio-venous difference studies) (Gordon and Cherkes, 1956). In 1957, Wadström observed that 90 minutes after the injection of 0.01 mg. of epinephrine into rabbits there is a decrease in the triglyceride content of the fat depots associated with corresponding increases in the levels of glycerol, mono and di-glycerides. This evidence pointed to the conclusion that epinephrine activates lipolysis or, what amounts to the same thing, inhibits resynthesis of triglyceride in the adipose tissue. More recently, it has been shown by Gordon and Cherkes (1958) that the addition of epinephrine to surviving adipose tissue, *in vitro*, accelerates the rate at which this tissue releases **Nefa** into the surrounding medium.

Shafir et al. have pointed out two interesting facts in relation to the effect of epinephrine on lipid metabolism. In the first place they observed that this hormone gives rise to parallel increases of **Nefa** and lipiprotein which latter plasma constituent is, in all probability, formed by the liver in response to the increased availability of fatty acids. The second, and perhaps the more intriguing, observation made by this group is that epinephrine exerts a more rapid effect upon lipid than on carbohydrate metabolism. Thus it is found that the hormone gives rise to a primary elevation of **Nefa** levels in plasma which gradually return to normal as the blood glucose rises. This observation is in agreement with the earlier results obtained by the same group in 1959 which indicated that the **Nefa** response to epinephrine can be prevented by simultaneous administration of glucose and insulin. The observation finds confirmation also in the work of Goldfen and Havel which showed that the **Nefa** response to norepinephrine, administration of which does not lead to marked hyperglycaemia, is sustained for a much longer time than that produced by epinephrine itself. The interesting fact emerging from these results is simply that, in time of stress, it is the lipid stores on which the body depends as a primary source of energy.

The second endocrine organ which exercises a profound effect upon lipid mobilization from storage sites is the anterior lobe of the pituitary. Some four hormones of pituitary origin have been shown to exert control over fatty acid metabolism; these are somatotrophin (growth hormone), thyrotrophic hormone (TSH), the corticotrophins (ACTH) and prolactin. It should be emphasised that the effects of ACTH and TSH are of peculiar interest in this connection, since, on the basis of *in vitro* studies, it is known that their action is not dependent upon the presence of their "target organs", though, in the whole animal, the presence of these organs will modify the lipid response observed.

The evidence so far to hand suggests that all four hormones influence lipid metabolism in essentially the same way: somatotrophin, however, by a sort of historical accident, has been studied most intensely and so the effects of this hormone will be considered at greater length than those of the other three.

In 1944, Stetton and Salcedo, injected anterior pituitary extract into mice, a procedure known to give rise to a condition of "fatty liver": they discovered by means of prior deuterium labelling that the excess hepatic lipid had been transported to that organ from

the adipose storage depots.

The problems involved in the isolation of pure and homogeneous preparations of the various pituitary principles made further progress in this field difficult and even today, render interpretation a matter open to doubt. However, in 1953, Greenbaum and McLean reported that treatment of rats with "purified" somatotrophin caused a very rapid increase in hepatic triglyceride content. In view of the fact that the hormone is known to increase the rate of fatty acid oxidation in the liver, and bearing in mind the results of Stetton and Salcedo above, it seems apparent that the accumulation of lipid material in the liver under these circumstances must result from an increased rate of transport of fatty acids to this organ from the depot fat. That somatotrophin can increase the rate of mobilization of **Nefa** from adipose tissue is further suggested by the finding that the plasma **Nefa** level of fasting dogs, already high, may be readily increased two-fold by the injection of "purified" bovine somatotrophin.

The *in vivo* evidence of a fat mobilizing action of somatotrophin is thus fairly clear cut, but unfortunately it has so far proved impossible to demonstrate this activity of the hormone *in vitro*. In 1958, however, White and Lugel in experiments with ACTH showed that a) corticotrophins are more active than somatotrophin in promoting fatty acid liberation *in vivo* and b) that they also display a powerful effect in the case of adipose tissue *in vitro*. On the basis of these observations, White and Lugel proposed the possibility, which still awaits experimental verification, that somatotrophin may be inactive in itself but that, in the intact animal, it may be metabolised to a product which is capable of promoting fatty acid liberation from the fat depots — the analogy here to the case of L-thyroxine is so obvious that no further comment is required.

The two remaining hormones, TSH and prolactin, are not so well defined as regards their action on lipid metabolism. For example, in vitro experiments have indicated that TSH is capable of increasing the rate of **Nefa** release from adipose tissue but only when present in unphysiologically high concentrations. The state of our knowledge concerning the action of prolactin on fatty acid metabolism is equally unsatisfactory. Reiss (1947) maintains that injection of this hormone leads to well marked depletion of the fat stores, but, as in the case of somatotrophin, no *in vitro activity* can be demonstrated. On purely teleological grounds, Reiss proceeded to argue that it is logical to expect prolactin to exercise a fat mobilising effect, especially in view of the fact that Shaw and Petersen (1938) have claimed, on the basis of A/V lipid differences across the lactating udder, that more than enough circulating lipid, in what form they do not say, is abstracted from the blood by this organ, to account for the entire lipid content of the milk. This effect of prolactin was also claimed on the basis of Houssay's observation that somatotrophin and the corticotrophins can, at least in part, replace prolactin in the maintenance of lactation in the hypophysectomised animal. A warning must be given at this point concerning the validity of such "round about" arguments however, and the whole status of the reliability of observations based upon work with so called "pure" anterior pituitary principles must be examined critically before acceptance.

We may now pass on to a consideration of insulin. Both the concentration and turnover of **Nefa** in blood are strikingly influenced by this hormone. In the normal fasting animal and in the diabetic animal, the circulating **Nefa** level is decidedly increased and indeed, in severe ketotic diabetes the molar ratio of fatty acid to serum albumin may exceed seven, which is the maximum number of fatty acid molecules which can be tightly bound by one molecule of serum albumin, the normal value being rather less than one (Goodman and Gordon, 1958).

In 1958 Dole observed that the administration of insulin causes a marked fall in the plasma **Nefa** level of normal individuals. As in the case of epinephrine considered above, the action of insulin is of particular interest in that this hormone has been shown (Dole 1958) to exercise an effect more rapidly on circulating lipid than on blood glucose. Once again, this may be regarded as a reflection of the importance of lipid metabolism in the living animal.

In addition to the *in vivo* findings outlined earlier, Cherkes and Gordon, in 1958, measured the rate of release of **Nefa** by epididymal fat bodies obtained from fasting rats, when these adipose tissue fragments were incubated in a medium containing bovine serum albumin as an acceptor of **Nefa**. In the absence of the hormone it was found that the tissue release 1.57 μm of fatty acid per gram per hour while, with the addition of physiological concentra-

tion of insulin a net uptake of Nefa from the medium was observed, amounting to 1.03 um per gram per hour. In other words the tissue which was releasing Nefa, on the addition of insulin, was persuaded to take up Nefa from the environment.

Other hormones have been reported to modify the metabolic activity of adipose tissue but their effects remain, in general, poorly understood and in the interests of brevity they will not be discussed here.

We have seen, then, some of the ways in which the uptake and liberation of fatty acids by adipose tissue may be controlled in vivo, but two very fundamental questions now present themselves. Firstly, what is the nature of the stimulus which causes the control mechanism to come into play and secondly, how is the neuro/humoral information translated into terms of biochemical process? To neither of these questions, particularly the latter, can definitive answers be given at this time but in what follows, some attempt will be made to clarify the situation insofar as it is possible, presently, to do so.

If we consider the normal physiological situation obtaining in an organism, then it is clear that the effect of somatotrophin on lipid metabolism, in the normal adult, may be assumed to be more or less negligible, though it may be significant in the young animal in which the provision of adequate amounts of oxidisiable substrate to the growing tissue is mandatory. In any event, it seems more likely than not that any physiological effect attributable to somatotrophin will be tonic in nature and not subject to rapid or marked fluctuation. On the other hand, all of the other components of the control mechanism are capable of dynamic variation dependent on tissue requirements from moment to moment. Thus, the mobilisation of **Nefa** in response to sympathetic nervous stimulation and to the release of epinephrine by the adrenal medulla is a biochemical reflection of the sensitivity of the nervous system to various types of stress, and is mediated by the activity of the higher nerve centres. Insulin, which strongly inhibits liberation of **Nefa** from depot fat and, as we have seen, may actually promote fatty acid uptake by adipose tissue, is liberated from the pancreas in response to the stimulus of high blood sugar levels acting directly upon the pancreatic cells. As I have said before, this provides an example of the close correlation existing between carbohydrate and lipid metabolism and explains the observed dependence of plasma **Nefa** levels on the nutritional state of the animal. Thus, a fasting animal may be expected to have a low blood glucose level associated with a high level of circulating **Nefa** but if an alimentary hyperglycaemia is established, the resulting increase in the level of circulating insulin may be held to explain the rapid fall in plasma **Nefa** which is observed in such a situation.

The mechanism by which this final control is actually mediated is rather more obscure. Throughout this article, reference has been made to the lipolytic activity of adipose tissue. The implication has been that the various components of the neuro-humoral control system modify the activity of tissue lipases. Clearly, however, an exactly parallel situation would arise if control was exercised by modifications in the rate of synthesis of triglyceride within the adipose tissue cells.

Let us examine these possibilities. First, the lipase hypothesis. Korn and Quigley found in 1957 that the only lipase activity demonstrable in many types of adipose tissue was that due to heparin activated "lipoprotein lipase". For this reason, a great, and perhaps disproportionate, number of investigators have studied the behaviour of this enzyme in relation to fat mobilization and deposition. Typical of this field of endeavour, is the work of Hollenberg (1959) who reported that the addition of glucose and insulin to adipose tissue from fasting animals, restores the capacity of the tissue, *in vitro*, to liberate "lipoprotein lipase" in response to heparin. From this he concluded that the enzyme may be concerned in the accumulation of fat in depots, possibly by influencing the rate of incorporation of lipoprotein fatty acids into adipose tissue cells. Many further studies have been undertaken and, by and large, all implicate lipoprotein lipase in the uptake of fat, in the form of **Nefa**, by the depots.

What then of **Nefa** release: in this case the substrate is rather different—not lipoprotein as in plasma, but more or less pure neutral fat (triglyceride) a fact that appears to rule lipoprotein lipase activity out of court since the activity of the enzyme toward pure triglyceride is vanishingly small. We must turn, then, to consideration of the second hypothesis, that dealing with rates of reesterification.

In 1960, Wood et al. pointed out that in rat epididymal fat tissue, glucose carbon is incorporated into the glycerol moeity of triglyceride at a rate many times greater than the

carbon of glycerol itself: they went on to demonstrate that adipose tissue homogenates require. L-α-glycero-phosphate or one of its phosphorylated precursors for optimum esterification of carbon labelled palmitate, glycerol itself being inactive in this system. If this is equally true of intact adipose tissue, then esterification of **Nefa** should be limited by the rate of formation of L-α-glycero-phosphate from glucose via di-hydroxy-acetone-phosphate.

Now it is known that the triglyceride of adipose tissue undergoes constant hydrolysis and re-esterification within the tissue; by what mechanism the hydrolysis is effected is not understood. However, it must be assumed on this evidence that, in the steady state condition, where there is no net uptake or release of **Nefa**, the liberated fatty acids are rapidly re-esterified. This re-esterification requires the presence of a supply of l-α-glycero-phosphate obtained by the metabolism of glucose via the Embden-Meyerhof pathway. The equilibrium situation between triglyceride and **Nefa** within the cells is quite obviously dependant upon a ready supply of glucose. During periods of hypoglycaemia, the **Nefa** of the adipose tissue might be expected to rise and since intracellular and extracellular **Nefa** are in a free translocation relationship this will lead to their liberation into the blood; conversely, after glucose and insulin administration the enhanced respiratory activity of the adipose tissue cells will ensure a plentiful supply of dihydroxy acetone phosphate and therefore of L-α-glycero-phosphate so that the fatty acid level of the circulating fluid will diminish as **Nefa** is withdrawn to be esterified within the cells.

This hypothesis has been found to accord well with experimental data in which the percentage of glucose carbon incorporated into the glycerol moiety of neutral fat has been estimated under varying conditions of availability of glucose. It provides an elegant example of the indirect way in which hormonal substances may modify cell metabolism since the action of insulin on fatty acid mobilization appears in this way to be explicable in terms of a primary action of the hormone, notably that of influencing the translocation of glucose across cell membranes. Attractive though the hypothesis may be, however, a note of caution must be sounded—the energy transformations and relationships in systems such as this remain obscure, complicated as they are by the very peculiar solubility properties of the participating lipids and lipoproteins and estimations of equilibrium constants for such systems have not, so far, been attempted.

In this article a very few of the advances which have been made towards the understanding of adipose tissue and its role in lipid metabolism have been discussed. We have considered the recognition, some thirty years ago, of the fat depots as dynamic entities, the discovery of the significance of **Nefa** in metabolism and the possible means by which the control of lipid uptake and release by adipose tissue may be affected under physiological conditions.

What has emerged is still a far from coherent story and we can only wait, perhaps for a further quarter of a century in the hope that the final word may yet be spoken.

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