Non-Esterified Fatty Acids in the Blood of Obese and Lean Subjects

EDGAR S. GORDON, M.D.*

It is a common belief of physicians and laymen alike that basic differences in energy metabolism must exist between obese and thin human subjects. Despite the failure of biochemists and physiologists to identify any definite anomaly, the belief has persisted that a higher over-all thermodynamic efficiency of obese persons could explain their apparent low requirements of energy and the resistance to weight loss which becomes manifest when they are subjected to a sustained caloric deficit. The report herein deals with investigations bearing upon this problem as a result of the observation that the pattern of fat mobilization and utilization under fasting conditions is demonstrably different in fat and thin subjects.

Non-esterified fatty acids (NEFA) have been measured by the titration method described by Dole. Venous blood is drawn at intervals throughout the day, using heparin as the anticoagulant. It is processed immediately by centrifugation and separation of the plasma, which is then extracted with heptane. Final titration with dilute sodium hydroxide is performed in a two-phase system agitated with a stream of nitrogen, using bromthymol blue as an indicator. All subjects are fasted throughout the test; the first blood sample is drawn at 8:00 A.M. after an overnight fast and the last specimen drawn between 4:00 P.M. and 5:00 P.M., so that the test period represents the last nine hours of a twenty-four hour fast. Quiet activity about the ward is allowed and drinking water may be taken as needed. Normal healthy subjects of average body build show a progressive rise in NEFA concentration in the blood during the test. The curves in Figure 1 represent the changes in four normal subjects, two men and two women. Values obtained in the early morning after the overnight fast are usually between 300 and 500 μEq./L and rise during the ensuing nine hours to between 800 and 1,200 μEq. L. For unknown reasons, women tend to have somewhat higher values than men. Subjects, who are constitutionally thin, usually hyperkinetic and who are able to consume high caloric diets without any tendency to gain weight, show much steeper rises in blood NEFA values while fasting. Figure 2 illustrates values obtained for a twenty-five year old woman who is healthy, but literally unable to gain weight despite an excellent appetite. During four hours of fasting, the concentration of NEFA rose nearly 2,000 μEq./L and then fell precipitously 1,500 μEq./L when carbohydrate was ingested. These changes resemble somewhat the rise which accompanies strenuous physical exertion in normal subjects. The three curves shown in Figure 3 are from the same normal male subject: one, a control experiment; the second, illustrating the changes in concentration of NEFA induced by a diet very high in fat and the third, showing the extremely sharp rise brought about by vigorous exercise under fasting conditions. Obese subjects, on the other hand, show markedly elevated levels in the early morning which tend to remain essentially flat curves without any consistent trend upward or downward during the course of the standard test period. Figure 4 illustrates the typical flat curves in two fasted obese patients

From the Department of Medicine, University of Wisconsin Medical School, Madison, Wisconsin.
*Professor of Medicine.
NEFA in Blood of Obese and Lean Subjects

Fig. 1. Changes in NEFA levels in the blood, induced by fasting in normal subjects of average build.

Fig. 2. Lipid changes in thin subjects during fasting: a twenty-five year old woman weighing ninety-five pounds; a twelve year old boy weighing forty-seven pounds.

Fig. 3. The effects of strenuous physical exertion and a high (90 per cent) fat diet in a normal male subject.

Fig. 4. Changes in lipid levels in marked resistant obesity during fasting. ● = twenty year old woman weighing 290 pounds. ○ = twenty-two year old man weighing 380 pounds.

a man and a woman. Figure 5 represents the changes in NEFA, blood ketones and glucose in two other obese subjects, both women. Here again, there is a conspicuous absence of the consistent, progressive rise seen in subjects of normal weight. Note the respiratory quotient of 0.78 in one of these women.

Certain obese subjects, who are clearly overweight because of constant excessive food intake, show NEFA curves that seem intermediate between the flat plateau and the usual steady rise. Such patients lose weight quite easily when caloric intake is restricted. Figure 6 shows, on the left, the NEFA curves of...
Gordon

three patients, all of whom by history and response to diet were obese because of overeating. The curves show some tendency to rise, although the gradient is less than in normal subjects, and the values are all distinctly elevated. These women all lost weight easily when they were subjected to a sustained period of caloric restriction. On the right, curves from four "resistant obese" subjects show the characteristic flat curve at an elevated level; all had very small appetites, and none of these subjects lost weight even during observation in the hospital for prolonged periods of time.

Interpretation of these changes in concentration of NEFA, in the past, has been entirely in terms of changing rates of fatty acid mobilization from adipose stores, and the implication accordingly has been that fasted obese subjects do not mobilize fatty acids. Because of the dynamic nature of lipid metabolism, however, and especially because of the highly labile state of the NEFA fraction, it seems possible that the circulating level at any moment represents an algebraic summation of the rate of delivery from fat stores to the circulation and the rate of peripheral utilization of fatty acids for metabolic needs. Under these conditions, a rising level might be due to an accelerated rate of mobilization, a decreased rate of utilization, or both. Thus, a failure of consistent change in concentration of NEFA over a period of many hours, while it could be attributed to a failure of mobilization, might also be interpreted as a rapid rate of mobilization balanced by an equally rapid rate of utilization. Suggestive evidence that the latter might, in fact, be more correct was provided by the measurement of respiratory quotients which we, as well as others, have noted in obese subjects to be consistently below 0.8 and usually in the vicinity of 0.7. It is well known that such readings have classically been interpreted as indicative of predominantly fat oxidation.

Fig. 5. Lipid changes in marked resistant obesity during fasting. •—• = nineteen year old woman weighing 328 pounds; x-x = twenty-three year old woman weighing 215 pounds.

Fig. 6. Fasting blood NEFA curves in severe obesity.
Thus it appears that obesity is characterized by an increased, rather than a decreased rate of fat utilization, as might initially be suggested by the experimental data. The resistance of fat people to the development of ketosis, however, seems difficult to reconcile with these other data. Figure 7 illustrates the sharp separation of obese from normal subjects in regard to the ketosis produced by a high fat diet.\(^2\) We have repeatedly confirmed this finding and we believe that the phenomenon is probably due to some enzymatic mechanism in the liver in obesity which may protect Krebs cycle intermediates from the attrition that usually occurs in the absence of the constant regeneration normally derived from metabolism of carbohydrates.

In order to obtain further information on the dynamics of these changes in NEFA, studies have been instituted to measure the rate of oxidation of \(\text{C}^{14}\)-tagged palmitic acid administered intravenously to human subjects, both obese and normal. These have not proceeded far enough to report in detail, but it is already apparent that fatty acid degradation to carbon dioxide and water proceeds vigorously in obese subjects and even appears to be more intense and more rapid than in subjects with slim body build. Data of statistical significance have not yet been accumulated, but if the early trends are maintained, it would appear that obesity, at least of the resistant type, is characterized by a predominantly lipid oxidation for energy purposes in the course of ordinary metabolic needs. Such conclusions would correlate satisfactorily with the other indices of fatty acid oxidation, such as the constant low respiratory quotient in obese subjects.

Two additional clinical observations are worthy of mention. In those obese subjects with the flattest type of NEFA curve during periods of fasting, the sensation of hunger is consistently very slight or absent in contrast to the rather intense hunger of many or most of the slim subjects. This difference is apparent in both long and short fasting periods. It also seems probable from these data that the ease of weight loss, when a caloric restriction is imposed and maintained, correlates with the NEFA curve, so that those with the flattest curves lose weight with difficulty or not at all, and those with gradually rising or near normal curves (those subjects, with obvious obesity from habitual overeating), lose weight very easily under these conditions.

REFERENCES

DISCUSSION
Dr. VINCENT P. DOLE (New York, New York):
I wonder whether Dr. Chalmers would care to ask any questions of his friends, and vice versa.
DR. CHALMERS: I would like to make a comment on Dr. Gordon's paper.

This characteristic of resistant obese patients in whom the NEFA levels do not rise with fasting, reminds us of patients we have observed and given ketogenic (usually 90 per cent fat, 1,000-calorie) diets. We find that it is extremely difficult to induce hypoglycemia and ketosis in very obese patients with diets of this kind.

I wondered whether perhaps there was some link in the mechanism for these two phenomena.

DR. GORDON: I am sure there is a link. We have had the same experience, but I have not been able to put it all together into a pattern.

Respiratory quotients at the end of a long fast in these fat people were constantly very low on the order of 0.75, 0.76, 0.77. This did not seem consonant with the hypothesis that they were not mobilizing fat because they were using some other fuel. If it were some other fuel, it must be carbohydrate. If they were using carbohydrate instead of NEFA, then there must surely be a rise in respiratory quotient. But there was none.

Therefore, it seemed to me that they were still living on fat, on their own fat and not on carbohydrate. What little evidence we have thus far would indicate that this is so. We are going to continue to experiment to discover if we can find out what the pattern of oxidation of fat is in these people.

We have two very bizarre observations on these resistant patients to whom we have given carbohydrate at the end of a long fast. Their respiratory quotient one hour later, instead of rising, fell further.

I do not think I have ever noted this before, and I have no explanation. It is almost as though the carbohydrate made them burn more fat.

DR. DOLE: Dr. Cahill, do you have any questions?

DR. CAHILL: No. I only want to comment on the extreme sensitivity of the mobilization effect of your factor, Dr. Chalmers. I think this is fascinating. It is down to 1 mg. per ml. of what still is probably a crude protein fraction. This is almost as active as ACTH and more active, certainly, than growth hormone. I think this is striking and very important.

DR. GORDON: Have you, by any chance, had occasion to examine the urine of patients who might fall into this category, who are obese and who are not able to lose weight?

DR. CHALMERS: Yes. I think, as far as we go, we do not detect any difference between the amounts obtained from obese patients and non-obese patients, but we have not really looked into this critically enough, because our assay methods are not very quantitative. I think this needs to be confirmed, but so far we do not really detect very much difference. We certainly get a great deal of activity in the urine of obese patients.

DR. DOLE: I now would invite general questions from the floor.

DR. LILLIAN RECAN'T (St. Louis, Missouri): I would like to ask a little bit more about this fat-mobilizing material in the urine. I wonder whether you have had any opportunity to look for any other metabolic effects of this material. For example, have you attempted to find out whether it has any of the patterns of growth hormone activity when injected into an animal? Does it produce growth? Has this been tested? Does it have any effects at all on, let us say, cardiac glycogen in the fasted animal? Is there any evidence of any of these activities? I understand that you have tested for the ACTH activity.

DR. CHALMERS: We have not studied cardiac glycogen, but it always causes loss of weight even in quite young animals when given for periods up to twenty-one days. So I think we can say it does not cause growth.

DR. JAMES M. SALTER (Toronto, Ontario, Canada): Apropos of Dr. Recant's question, one of the hypotheses, of course, has been that growth hormone acts by increasing the mobilization of fat and making more energy available for synthesis of protein and that the growth is an indirect outcome of this action.

I think the whole thing is rather interesting because of Dr. Chalmers' fat-mobilizing substance. Obviously there is an increase in the utilization of fat with no retention of nitrogen at all. And it seems there are a number of other things, like adrenaline and others and even vasopressin, which increase the utilization of fatty acids without any effect on nitrogen metabolism.

DR. CAHILL: I think this is true for all these factors. There is something unusual going on here. I mean, conservation of energy has to hold. In other words, if you have an animal that eats just a little bit less but still has a marked weight reduction, you cannot explain this just because NEFA moves out of adipose tissue and goes somewhere else in the body. Unless that energy is oxidized, some other process is going on.

The only possible explanation for the expenditure of energy has got to be in the basal metabolic rate. So I think you have to assume that these animals have an increased respiratory rate, just as do your glucagon-treated animals, Dr. Salter.

DR. GORDON: This phenomenon of people who do not lose weight is really the most tantalizing thing that confronts physicians. There are these basal people who can live on 600 calories and not lose any weight. On what are they surviving? If we measure their basal metabolism in terms of calories, we get figures in excess of 600 calories per twenty-four hours. It would seem that on this diet they are in a caloric deficit all the time, but still are not losing any weight.

I am still an admirer of the laws of thermodynamics, but these people seem to be thermodynamic paradoxes, and I certainly would like an explanation.

DR. DOLE: Does anybody have one?

DR. F. X. HAUSBERGER (Philadelphia, Pennsylvania): I would like to mention experiments which were made during World War I and II, especially during World War II, on obese people. There were certain types, who (when their food intake was very much restricted, say to 600 calories and even below) for quite a long time did...
not lose weight, but then very suddenly lost weight. That weight loss then was water.

I think these people (whom you describe) on a diet of about 800 calories, retain some water, because there is no other possibility if, as Dr. Cahill pointed out, we believe in the laws of energy.

**Dr. Gordon:** This point, supposedly, has been investigated by a measurement of total body water in these people, and it has been found to be normal or very slightly decreased. This is not our work, but it has been reported.

I think what we are all looking for, unquestionably, is something that controls efficiency. If any one of us in the room were to suddenly increase the efficiency of his machine by 1 per cent and all other factors in his life remained the same, he would immediately start to gain weight, but could accomplish the same amount of useful work with 1 per cent less expenditure of energy.

It does not take a large magnitude of change in efficiency to account for this sort of thing. But, to my knowledge, nobody has ever isolated any point in any metabolic scheme in which the efficiency might be changed, except for those instances like dinitrophenol or, possibly, thyroxin, where there is an uncoupling of P to O ratios.

**Dr. R. H. Williams (Seattle, Washington):** Dr. North and I have carried out some studies a little like the one Dr. Gordon executed in regard to the fasting of these subjects. The NEFA levels of normal subjects, as you demonstrated, went up. The obese ones were somewhat like that, although, as you indicated, there were variations among different subjects.

I do not believe you made a comment, but I believe that your figures indicated that your obese subjects were also at a higher level than the normal early ones. This is something that puzzled me, and I have not quite understood that. It seems as though the obese subjects were failing to mobilize NEFA for some reason or under certain conditions they had more not being utilized.

The other point was that when we administered epinephrine, the values in normal subjects increased more than did the obese ones. Again, there was a defect in the mobilization.

**Dr. Gordon:** Dr. Dole, I recall that you were the first one who showed that the obese subjects have a higher fasting level. Why is this? Do you have any ideas?

**Dr. Dole:** No, I do not. I thought, in the original interpretation, that there was some doubt as to how long the fat person had been functionally starved. At that time I had not thought of the possibility that the fat person might fail to rise, and it occurred to me that if I waited until, let us say, eight in the morning, after a night’s dinner, to take blood out of a thin and a fat person, the fat person might be somewhat in the position that the thin person would be in at 10 A.M. I had thought that perhaps the time of starvation represented just a different functional interval for the two people. Now, of course, it comes into somewhat more question.

**Dr. Max Kleiber (Davis, California):** I cannot get excited about the law of conservation of energy in this particular case, because so far I have failed to see an explanation of the necessity for the energy in basal metabolism for the vital functions.

Borsok made a calculation of the renal function, and he came to the conclusion that the kidney has a very high capacity for work with an extremely low efficiency. So the work of the kidney, certainly, cannot explain the basal metabolism. Neither can the work of the heart, nor the work of the muscles of the thorax.

I really do not see why you suspect that there is something wrong. Apparently, a good deal of the basal metabolism is just absolute waste. It is not a necessity for work, because the work of an animal can be rather highly efficient—it can be 25 per cent, 30 per cent efficient—and if he has that efficiency, then I do not see why an animal or a human being cannot exist on 600 calories instead of 2,000. Why should he have 2,000 calories unless he is in a cold environment? Then I could understand it. But that is another question.

Do you have those people tested in a cold environment, so that they give off that much heat?

**Dr. Gordon:** If you are directing that at me, no, we have not. But I daresay that almost anyone in this room, if he subsisted on 600 calories per day, would very rapidly lose weight. I am sure I would. I don’t know why, except that I always thought I was in a caloric deficit when I was getting that amount of food.

**Dr. Dole:** I think that perhaps some work we have done which has not been published might contribute a bit to that. It is a fact that when a person reduces by a caloric deficit, the metabolic rate does, indeed, decline very much. Obviously, the calories burned come out as heat, because the body temperature stays constant.

The question as to whether one is in caloric balance at 600 calories needs a little closer examination just in terms of method. You realize that if you expend only 10 gm. of fat per day, you get ninety calories. So a few hundred calories can be made up of what is almost a trifling amount of body weight, and that can easily be masked by a small amount of water or indeed just by the noise and inaccuracies in your weighing unless you have a much more accurate scale and a much more precise metabolic control.

The notion that people have controlled this by measuring body water is, I think, to some extent an illusion. You have 60 to 65 per cent body water. In a person who weighs eighty kilograms, there is a great amount of water and we are trying to detect the difference of a few grams. The methods, even if you give them 3 per cent or 5 per cent accuracy, are in the order of magnitude of a hundred times more uncertain than the small changes in water you are trying to reconcile.

No measurements of body water are capable of discriminating between those little fluctuations that could replace a caloric equivalent of fat that would cover a deficit of a few hundred calories.
When one says that the fat person on a 600-calorie diet fails to lose much, if any, weight over a period of a month or six weeks, it still does not mean that the caloric expenditure is as low as 600. I think it might be 1,200 or 1,000. There is a marked decrease, in other words, but it need not be such an extremely low level as to be undetectable by the methods that are available.

**DR. THEODORE B. VAN ITALLIE (New York, New York):** I would like to raise a few questions that have troubled me in this discussion.

The assumption seems to have been made that the level of NEFA in the plasma somehow represents an indication of the rate of fat mobilization, which may be true under certain circumstances. But, after all, it is only a level, and fatty acids are not only being mobilized but also being disposed of. Certainly the level can be altered or changed by rate of utilization as well as rate of mobilization. If rate of utilization increases for some reason, there is no reason to suppose that the level would necessarily rise. Indeed, I sometimes wonder why it does rise at all.

The second thing that I wanted to bring up was the assumption that seems to have been made by several of the speakers today that in obese patients with diabetes there is a deficiency of insulin. From my conversations with Dr. Renold on his work with insulin-like assay procedures and with Dr. Mayer on his analyses of insulin in obese hyperglycemic mice, there is very little evidence that obese patients with diabetes are, indeed, deficient in insulin, at least as we ordinarily attempt to measure this substance.

The third point which is, perhaps, the most confusing, is the question of epinephrine. I believe Dr. Cahill said that epinephrine promoted the entrance of glucose into the adipose cell. I find this difficult to reconcile with some of the other data that have been given on the effect of insulin on uptake of glucose by rat diaphragm. Groen has demonstrated, I believe, that epinephrine is a powerful inhibitor of insulin activity as far as the rat diaphragm is concerned. Dr. Ingle has shown that in the eviscerated rat, epinephrine inhibits the rate of glucose disposal. Somogyi has shown that epinephrine impairs peripheral arteriovenous glucose differences. And the Drs. Cori, to mention another approach to this experiment, have shown that with perfusion of glucose through the hind limb of the dog, when epinephrine is given the rate of utilization of glucose is decreased. I wish that someone could clarify just what epinephrine does in the periphery.

**DR. CAHILL:** You quoted the work of Dr. Ingle. The paper was by Ingle and Nezamis. On looking at the paper, he at times got an increased uptake of glucose in the extremity or in the eviscerated preparation. This was startling to him. He quoted this in his paper; occasionally there was no question but there was an increased uptake of glucose, and he also quoted some previous papers. This whole idea was reviewed by Griffiths in 1954 in *Physiological Reviews*—there are isolated experiments where it was found that epinephrine did, indeed, cause a glucose uptake.

Going over some of these experiments, these were experiments across a hind limb using, perhaps, the major vessels, which included the inflow of the sapheous. This makes one wonder whether epinephrine does cause a decreased glucose uptake in muscle and possibly an increased glucose uptake in adipose tissue.

One can offer a very brief explanation. The classic theory, of course, for the inhibition of glucose uptake by muscle is that in muscle, when you have glycogen breaking down to glucose-6-phosphate, glucose-6-phosphate being an inhibitor of hexokinase, the phosphorylation of glucose is inhibited. Parker and Kipnis have both demonstrated that when muscle is treated with epinephrine, there is an increased glucose concentration inside the cell but yet a decreased metabolism of glucose.

Now look at adipose tissue. Adipose tissue from the normal animal, as Dr. Tuerkischer and Dr. Wertheimer, I believe, demonstrated in 1942, has no glycogen, or a barely detectable amount. It is about 0.01 per cent as determined with the finest techniques.

If epinephrine broke down this very small amount of glycogen, there certainly would be no inhibition of hexokinase by an accumulation of glucose-6-phosphate.

There is one other isolated discovery which was pointed out to me by Dr. Sutherland: if one increased the glycolytic rate in muscle by adding an oxidation-reduction mediator, in this case hydroxyquinoline; if one treated muscle with hydroxyquinoline and then administered epinephrine, that muscle exhibited an increased glucose uptake or increased glucose metabolism, which makes one wonder whether, on the cell wall, there is no epinephrine effect of inhibition. Once the possibility of glucose-6-phosphate inhibiting glucose phosphorylation is removed, epinephrine would then increase glucose uptake in muscle also.

**DR. HERVEY (Sheffield, England):** In relation to the obese person who eats very little, I should like to suggest that individual variations in energy expenditure should be considered. There are great variations, both in resting energy expenditure and in the energy required to do some defined task, such as walking at a particular speed. These variations are much greater than are sometimes realized when one looks at tables of normal metabolic rates.

Experience of the centuries has defined what is the normal food intake for a person of average energy expenditure: three meals a day with so many courses, and so on. If the person who happens to be low both in his needs for resting and activity is offered this, it is probable that if he eats what he is offered, he will tend to get fat.

There is a suggestion being made that there may be a feedback control of food intake, geared to the amount of fat in the body; the idea being that if excess fat accumulates this inhibits further food intake, so that the fat is held constantly and, incidentally, food intake matches expenditure.

If you had such a system, the effect would be that
as the person got fat as a result of his low need for energy, the accumulation of fat would gradually depress intake, and eventually balance would be re-achieved when the depression was sufficient to offset the amount of food he was being offered. His intake would, then, be back to his low requirement. The end result could be a person who was both fat and had a low requirement. In fact, the requirement might even be less than before because of the extra insulation, and the accumulation of fat would, in cybernetic terms, correspond to the load error of what I suppose we would call a lipostat rather than a thermostat.