Observations on the Peripheral Metabolism of Nonesterified Fatty Acids

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As Andres, Cader and Zierler[1] pointed out in 1956, although skeletal muscle represented some 40 per cent of the total body mass, and despite our wide knowledge of the metabolic activity of excised muscle and muscle extracts, we were surprisingly ignorant of the quantitative importance of various metabolic substrates in supplying the energy requirements of muscle in fasting subjects. After measuring glucose uptake, Andres and his co-workers found that its oxidation would account for only 20 per cent of the basal oxygen consumption of the muscles, and they suggested that lipids might play a major metabolic role. This view about skeletal muscle had some support from the investigations of Bing and his associates[12] who found an appreciable uptake of nonesterified fatty acids (NEFA) by cardiac muscle.

More recently it has been shown that: (1) NEFA were found present in very high concentrations in the blood even under fasting conditions; only 1 to 2 per cent of lipids were needed to account for all the unidentified substrate,[1] (2) there was observed a relative ease of transcapillary exchange of the NEFA fraction[1] so the fatty acids were efficiently taken up[3,14] and readily oxidized by skeletal muscle,[1,4,12] and (3) NEFA were found to possess a short half-life of two to three minutes, resulting in a turnover rapid enough to furnish most of the energy needs of the tissues for lipids.[3,15]

However, in none of these studies of skeletal muscle was the amount of blood perfusing the tissue measured. Thus, the main purpose of this investigation was to evaluate the role of NEFA in the metabolism of skeletal muscle in the fasting subject, measuring not only arteriovenous differences but also blood flows, thereby permitting calculations of NEFA uptake on a quantitative basis.

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METHOD

This study was done in conjunction with other investigations being carried out at the same time.[10] Subjects fasted overnight during which time any insulin treatment was omitted. All the studies were done in a hospital ward, the patients lying comfortably in bed. Arterial and venous blood samples were withdrawn simultaneously through fine polythene catheters which had been threaded through needles into the brachial artery and into an antecubital vein carefully chosen as draining the muscle compartment of the forearm. As the blood was withdrawn over a period of one minute, blood flows were measured with a venous occlusion plethysmograph. The concentration of NEFA, in mEq./L., of the arterial and venous samples, was determined by the Van der Vies modification of the Van de Kramer method.[11] All determinations were performed in duplicate by the co-author (G.S.) and were considered valid only when the titration error of each pair of samples varied less than 5 per cent.

The percentage of uptake of NEFA by the forearm muscle was calculated as:

$$\frac{(\text{mEq./L.})_{\text{art.}} - (\text{mEq./L.})_{\text{ven.}}}{(\text{mEq./L.})_{\text{art.}}} \times 100.$$

Eleven diabetic and seven control arterial and venous concentrations were obtained from fifteen patients. The tissue NEFA uptakes were calculated from the arteriovenous difference of the NEFA concentrations and the blood flow. The units used for tissue NEFA uptake were mEq./100 ml. forearm tissue/minute.

RESULTS

1. *Arterial and venous* NEFA *concentrations of diabetic and control patients.* Figure 1. A wide range of concentrations was evident in both groups, there being no specific NEFA level differentiating the diabetic from the control patients.

2. *Uptakes of* NEFA *by skeletal muscle of the forearm in diabetics and controls.* Figure 2. Significant differences in uptakes between diabetics and controls were noted. Patients with diabetes exhibited uptakes of NEFA

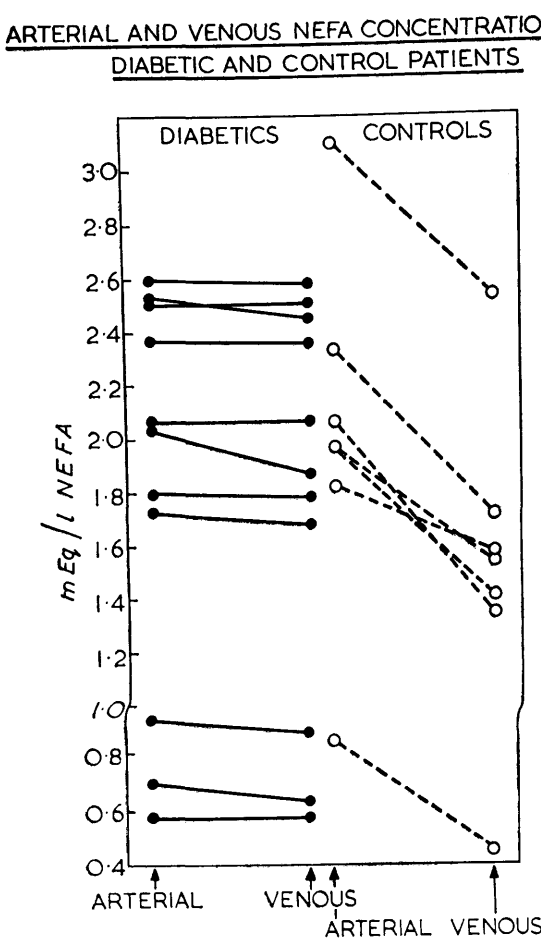

FIGURE 1

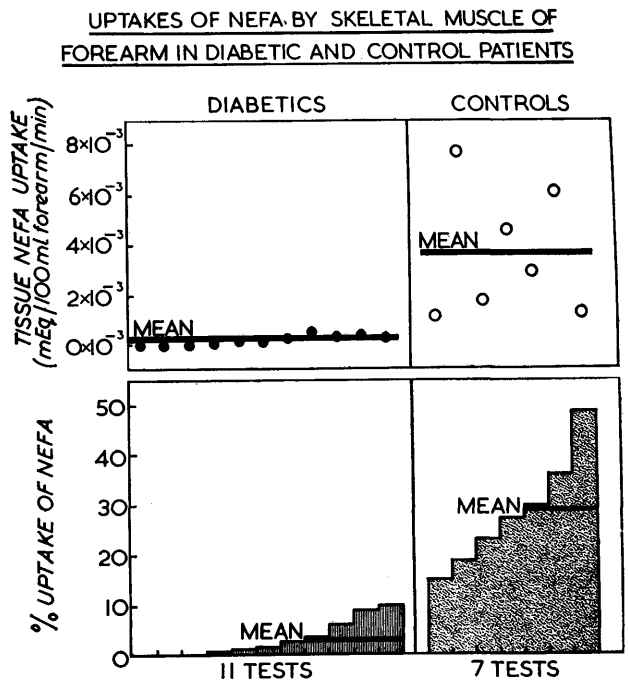

FIGURE 2

ranging from 0.0 per cent to 9.2 per cent, giving a mean of 2.9 per cent. In contrast, a much higher percentage of uptake was seen in the controls, who had a range of 14.2 per cent to 48.0 per cent, with a mean of 27.7 per cent. This mean percentage of uptake of the controls was in close agreement with the analysis of Gordon's[14] ten normal cases, in which the mean uptake was 26.9 per cent.

Correcting for blood flows, more noticeable differences were exhibited by the tissue NEFA uptakes. Diabetics showed a very minimal tissue NEFA uptake, averaging 0.17×10^{-3} mEq./100 ml. forearm/min., with a distribution of 0.00×10^{-3} to 0.44×10^{-3}. Again, the control patients' uptake was higher, ranging from 1.01×10^{-3} to 7.70×10^{-3} mEq./100 ml. forearm/min., a mean of 3.59×10^{-3}. The controls produced a wider distribution of tissue uptakes than the diabetics, who had a very limited range.

3. *Uptakes of* NEFA *by skeletal muscle of the forearm in severe and mild diabetes* (Figure 3). The role of insulin sensitivity and NEFA uptake was compared. Five diabetics were the juvenile type, whose diabetes was difficult to control and was considered relatively resistant to insulin. Three other patients (cases 3, 7, and 8) who had the mild, adult-onset type of diabetes not complicated by overweight, easily regulated and sensitive to insulin,[16] were compared to the severe cases.

The mild diabetics exhibited a higher range of percentage uptakes with a mean of 6.2 per cent as compared to 1.7 per cent uptake of the severe diabetics.

While the levels of tissue NEFA uptakes were equally distributed in both severe and mild diabetics, the latter cases had a mean tissue uptake (0.26×10^{-3}) twice that of the former (0.13×10^{-3}).

4. *The effect of insulin on* NEFA *metabolism in diabetes* (Figure 4). If the metabolic lesion of diabetes was related to inability of the forearm muscle to extract NEFA from the blood, then administration of insulin to a diabetic would be expected to correct and thereby increase the uptake of NEFA. This was the case in Patient No. 3.

Twenty-five minutes following the injection of 0.03 units of glucagon-free insulin, the diabetic's uptake increased from 0.9 per cent to 16.9 per cent, with a concomitant fall of the arterial NEFA concentration by 14.1 per cent.

5. *Repeated tests in two diabetics* (Figure 5). Over a period of more than three months, repeated tests in

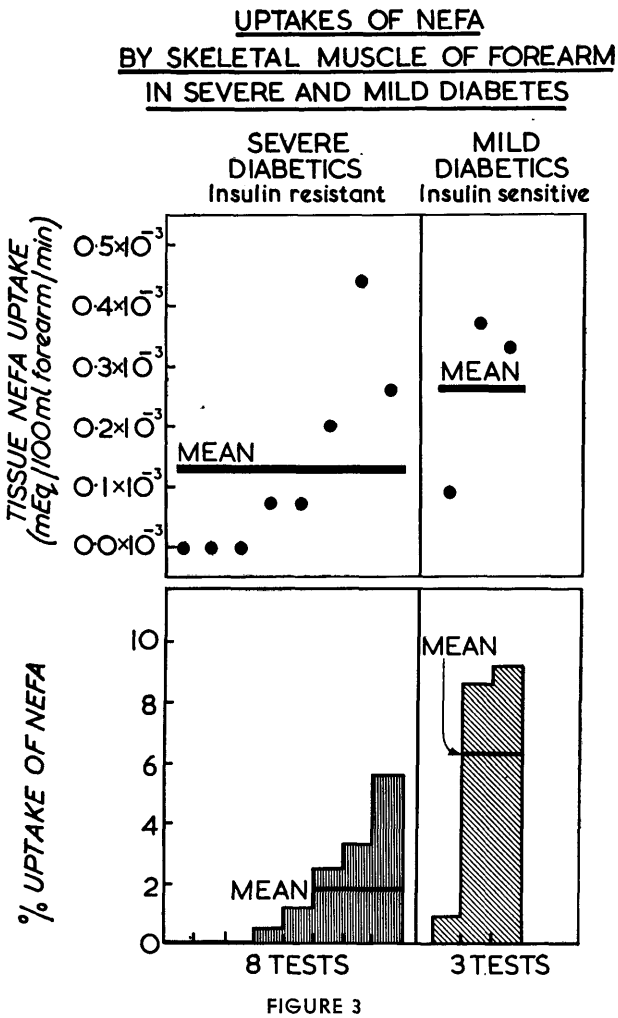

FIGURE 3

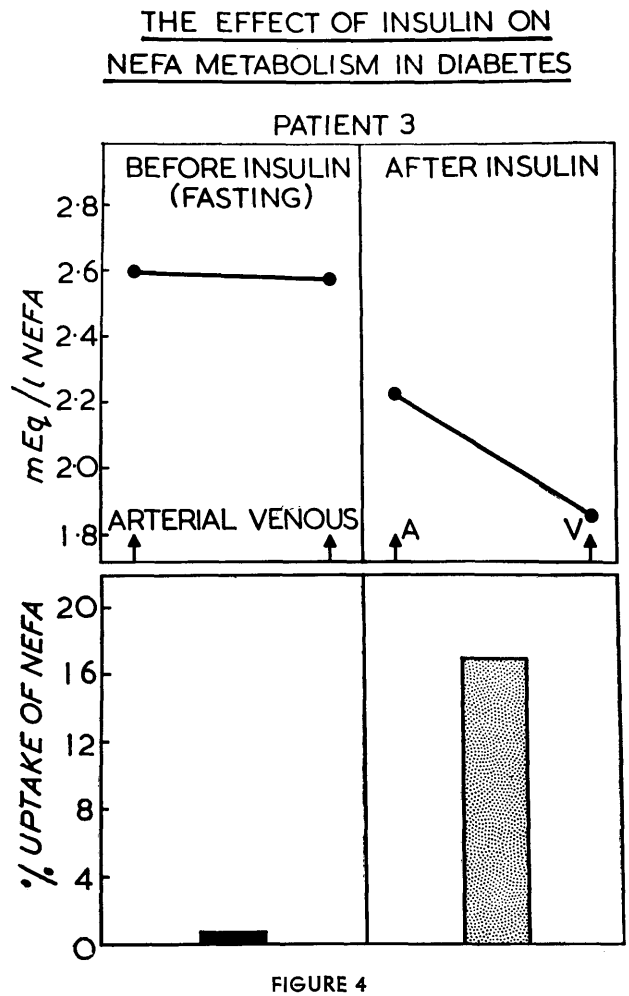

FIGURE 4

two diabetic patients showed an individual variation of NEFA concentrations. But the uptake varied less than 1½ per cent.

6. *Hepatic venous samples.* Patient No. 6, a diabetic, had an hepatic catheterization performed at the same time as the peripheral blood samples were withdrawn. The hepatic venous concentration (done in tripilicate) was 0.93 mEq./L., a value essentially the same as the peripheral venous concentration of 0.90 mEq./L.

DISCUSSION

The most likely explanation of the differences in uptakes between diabetics and controls was that normal skeletal muscle could withdraw and retain NEFA from the arterial blood for storage purposes, an ability not shared by the diabetic patients. To uphold this theory, one might cite past investigations:

1. Uptake of NEFA by skeletal muscle has been demonstrated for immediate energy needs (oxidation) or future use (storage).[8] Stadie[5] has shown that the enzymatic systems causing oxidative catabolism of fatty acids are unimpaired in diabetes. The deficiency of diabetes is the inability to synthesize fatty acids. Thus the metabolic defect in diabetes would be the anabolization of fats for storage[3,7,9] and not the catabolization of fats for oxidation.

2. The investigations of Dole[2,6] also implicate the storage depots of the body as the regulators of NEFA concentrations. In normal states of metabolism, Dole found proper utilization of carbohydrate inhibited the release of NEFA from storage depots. The diabetic patient, however, cannot control nor limit the output of NEFA from the tissue stores. Thus, Dole was of the opinion that inability to store NEFA was present in conditions of defective carbohydrate utilization, such as diabetes mellitus.

3. The diabetics' average percentage uptake of NEFA in this study probably afforded sufficient substrate for

REPEATED TESTS IN TWO DIABETICS SHOWING INDIVIDUAL VARIATION OF NEFA CONCENTRATIONS, BUT SIMILARITY OF % UPTAKE

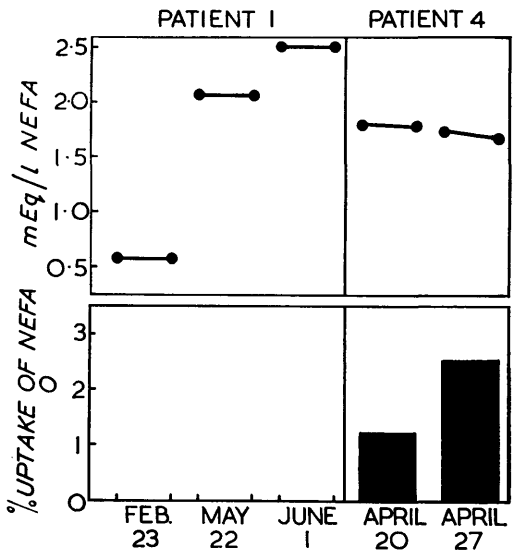

FIGURE 5

oxidation. Only 1 to 2 per cent of circulating NEFA was found necessary for immediate oxidation,[1] and the mean amount extracted by the diabetics here was 2.9 per cent.

Thus, our findings confirmed the previous investigations in which the differences of uptakes between diabetics and controls could not be explained by a deficiency of oxidation, but rather by a defect in the ability to take up and store NEFA.

In this study, other findings were of importance. First, as one progressed up the scale of insulin sensitivity, from severe diabetic to mild diabetic to control patients, an increase of the uptakes was seen. Whether this was related specifically to insulin sensitivity or to the ability to utilize carbohydrate due to other influences is undetermined. Dole[2] thought that insulin was not the sole regulator of NEFA metabolism. Second, even if other factors besides insulin govern NEFA, insulin is still an important influence. The effect of insulin on NEFA metabolism has been shown elsewhere,[2,3,6] as well as by this investigation.

TABLE 1

Case number	Initials, age, sex	NEFA (mEq./L.) Arterial	Venous	Per cent of uptake of NEFA	Blood flow	Tissue NEFA uptake units $\times 10^{-3}$
Diabetics						
1a	DS, 36 M	0.58	0.58	0.0	5.5	0.00
1b		2.07	2.07	0.0	5.8	0.00
1c		2.51	2.51	0.0	3.4	0.00
2	JH, 47 M	2.37	2.36	0.5	7.4	0.07
3	RD, 64 F	2.60	2.58	0.9	4.6	0.09
4a	MC, 30 F	1.80	1.78	1.2	3.5	0.07
4b		1.73	1.68	2.5	4.0	0.20
5	HW, 22 M	2.53	2.45	3.3	5.5	0.44
6	JT, 20 F	0.95	0.90	5.8	5.2	0.26
7	EH, 70 M	2.04	1.87	8.6	2.2	0.37
8	FP, 64 M acromegaly	0.71	0.64	9.2	4.7	0.33
Mean				2.9	4.7	0.17
Controls						
9	AT, 43 F obesity	1.82	1.56	14.2	3.9	1.01
10	GE, 20 F hyperthyroidism	3.10	2.53	18.1	13.5	7.70
11	LB, 26 F acromegaly	1.97	1.53	22.4	3.9	1.72
12	PS, 63 M	2.33	1.71	26.7	7.4	4.59
13	JC, 24 M	1.97	1.40	29.0	5.0	2.85
14	WL, 38 M acromegaly	2.06	1.34	35.2	8.4	6.05
15	KF, 35 M	0.87	0.45	48.0	2.8	1.18
Mean				27.7	6.4	3.59

SUMMARY

Using a technic for the quantitative study of the metabolism of peripheral tissue, we have investigated the uptakes of nonesterified fatty acids (NEFA) in seven control and eight diabetic patients.

The results showed that the arterial concentrations of NEFA were the same in the two groups. However, the mean tissue NEFA uptake in the controls was twenty times more than the diabetics.

The high uptakes of NEFA in the controls suggest these fractions of fat play an important role in the peripheral metabolism of normal skeletal muscle.

Also, the mild, adult diabetics exhibited a higher uptake than the severe, juvenile diabetic patients. Administration of insulin to the diabetic patient increased the uptake of NEFA from 1 per cent to 17 per cent.

It would therefore appear that the metabolic lesion in diabetes also includes failure of the peripheral tissues to take up NEFA, a fault which may be corrected by insulin.

SUMMARIO IN INTERLINGUA

Observationes Relative al Metabolismo Peripheric de Nonesterificate Acidos Grasse

Utilisante un technica pro le studio quantitative del metabolismo de histos peripheric, nos ha investigate le acceptation de nonesterificate acidos grasse in septe subjectos de controlo e in octo diabeticos.

Le resultatos monstrava que le concentrationes arterial de nonesterificate acidos grasse esseva le mesmes in le duo gruppos. Tamen, le acceptation medie de nonesterificate acidos grasse per le histos esseva vinti vices plus grande in le subjectos de controlo que in le diabeticos.

Le alte valores pro le acceptation de nonesterificate acidos grasse que esseva observate in le subjectos de controlo pare indicar que iste fractiones de grassia ha un rolo importante in le metabolismo peripheric de normal musculo skeletal.

In plus, diabeticos adulte con leve formas del morbo exhibiva plus alte valores de acceptation que diabeticos juvenil con formas sever del morbo. Le administration de insulina al diabeticos resultava in un augmento del acceptation de nonesterificate acidos grasse ab 1 pro cento usque a 17 pro cento.

Per consequente, il pare que le lesion metabolic de diabete include etiam le non-acceptation de nonesterificate acidos grasse in histos peripheric e que iste defecto pote esser corrigite per insulina.

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