CHAPTER 4

EFFECT OF EXERCISE ON THE FREE FATTY ACID LEVELS IN MUSCLE, LIVER, ADIPOSE TISSUE AND BLOOD IN THE PIGEON

It is now a well accepted fact that the breast muscles of flying birds utilize fat as the major fuel for energy during sustained flight. It has been estimated by George and Jyoti (1957) that at least 77% of the muscle metabolism in the pigeon during sustained muscular activity is due to the oxidation of fat. Similarly the heart is known to obtain its major energy requirements from fat (Visscher, 1938). Andres et al, (1956) found that the mean respiratory quotient of the forearm muscle was 0.8 and suggested that the major non-carbohydrate material which serves as the substrate for oxidation in muscle is lipid. Bing et al, (1954) have presented evidence to show that free fatty acids from the coronary circulation are extracted by the myocardium in the human heart.

Similarly in the case of flight muscles of birds the free fatty acids or the fat present in the muscle are not sufficient to give a continuous supply of the fuel to the muscle in prolonged activity as is the case in migration. Hence a regular supply of fat from the adipose tissue and liver to meet the high metabolic demands is to be expected.

Recent work from several laboratories have suggested that free fatty acids (FFA) of the plasma is utilized by the skeletal muscle during activity (Issekutz and Spitzer, 1960; Fritz et al, 1958; Carlson and Pernow, 1959; 1961; Friedberg

et al, 1960). It is well known that the FFA in the plasma are considered as lipids in transport from the adipose tissue to other tissues (Fredrickson and Gordon, 1958). Studies on the concentration of FFA in the circulating blood have given evidence to show that FFA in the blood are derived from the adipose tissue and that the amount in circulation and the rate of their production from the adipose tissue vary in response to the metabolic requirements of the animal (Gordon, 1956; Gordon, 1957; Gordon, 1958; Dole, 1956). Moreover, it is also known that the lipid release from the adipose tissue is influenced by certain hormones (Dole, 1956; Raben and Hollenberg, 1958; Hollenberg et al, 1960) and a variety of other chemical agents.

It was therefore thought desirable to investigate the level of FFA and its changes in the blood, muscle, liver and adipose tissue during rest and activity of the breast muscle in the pigeon. However, in the present studies, direct proof for the uptake of FFA by the muscle has not been tried, chiefly because of technical difficulties involved in obtaining blood from the muscle and also in measuring differences in arterial and venous bloods.

Materials and Method

Pigeons weighing from 300 to 320 gm. of either sex were used. After removing few feathers from the breast, the breast muscles were made to contract by stimulating them with an electronic stimulator. A convenient method was adopted for stimulating the breast muscles so as to avoid the excitement

and struggle of the bird during the process of electrical stimulation. The pigeon was blinded by covering the head with a black cloth mask during the stimulation of the muscle. It was found that by this blind-folding the bird was partially under some sort of a hypnosis and remained calm without any struggle. The muscle was stimulated at a rate of 5 stimulations per second with a duration of 2 milliseconds and a voltage of 20. After the muscle was stimulated for 30 minutes the blood was drawn from the heart with a syringe and collected in oxalate coated test tubes and refrigerated promptly. The blood was later centrifuged at 2500 r.p.m. for 5 minutes to separate the plasma. The plasma was poured into another tube and stored in the refrigerator. After collecting the blood, the animal was decapitated and a piece each of the breast muscle, liver and abdominal adipose tissue was separately taken, weighed quickly and dropped in the ethanol-ether mixture (1:1) for the extraction of FFA.

Control experiments were carried out on pigeons from the same group. In few cases the controls and the experimental samples were taken from the same animal. But in such experiments the animals did not live throughout the experimental period due to bleeding and hence all the values given in the results are of different animals for controls and samples.

The FFA level in the blood plasma was estimated according to the method described by Grossman *et al*, (1954; 1955). 1 ml. sample of the plasma was added by mixing with 1 ml. of 0.2 M phosphate buffer of pH 5.0 and 3 ml. of 95% ethyl alcohol was

then added and mixed well. 3 ml. of petroleum ether was added to this and the tubes were shaken for 1 minute and then centrifuged for 2 minutes. After centrifugation the petroleum ether layer was taken with a syringe and transferred to another 17 X 120 mm test tube. The extraction with petroleum ether was repeated twice more and the combined petroleum ether extract evaporated in a water bath at 70°C. 2 ml. of alcoholic thymol blue were added and the tubes were heated for 1 minute at 90°C and titrated with aqueous NaOH while the tube was still hot. A blank was run with each set of experiment. The FFA level in the blood is expressed as Meq. of FFA/litre of plasma.

The FFA content of the muscle, liver and adipose tissue respectively was estimated in the same way with slight modification. An aliquot of the alcohol-ether extract of the tissue was evaporated in a water bath so as to remove the ether. After cooling 1 ml. of the phosphate buffer was added to the remaining alcohol part of the extract and mixed well. The rest of the procedure was carried out as in the case of plasma FFA. The FFA levels in the muscle, liver and adipose tissue are expressed as Meq. of FFA/ gm. wet tissue.

Results

The results obtained are presented in Table 1. It is clear from the data obtained that there is a reduction of FFA in the blood, liver and adipose tissue after the muscle has been stimulated for 30 minutes. In the muscle on the other hand it was found that the FFA content was higher than in the controls.

In some cases the control samples for the resting muscle was obtained from the same animals used for the experiments before they were stimulated. In these cases too the pattern of increase in the muscle and reduction in plasma, liver and adipose tissue, was obtained. It is of interest to note that there is a significant uptake of FFA from the blood 1.58 Meq/ litre/ 30 minutes during the stimulation. In the muscle on the other hand a very high increase was observed. It should be however, mentioned here that the reduction in the FFA content of the blood observed may not be completely due to the uptake by the muscle alone but other tissues like heart, diaphragm, liver and kidney may also be extracting FFA from the blood.

Table 1

The FFA content of the blood plasma, muscle, liver and adipose tissue in the pigeon before and after exercising the breast muscle by electrical stimulation.

	Blood plasma FFA / Meq./ litre	Muscle FFA Meq./ gm. wet tissue	Liver FFA Meq. / gm. wet tissue	Adipose tissue FFA Meq./ gm. wet tissue
Resting	3.16 (8) ± 0.491	5.09 (8) ± 0.99	26.31 (6) ± 4.51	48.64 (7) ± 7.98
Exercised	1.58 (9) ± 0.48	8.93 (10) ± 2.02	14.41 (6) ± 1.90	36.29 (6) ± 7.88

Figures in the parenthesis indicate the number of animals used for each set of experiment.

Discussion

The presence of free fatty acids in plasma has been shown a centuary ago by Hoppe-Seyler (1883-1884). In recent years the metabolic significance and importance of FFA in energy metabolism has been realized from the extensive studies of several workers. In plasma,FFA is greatly associated with albumin (Kendall, 1941; Davis and Dubois, 1947; Cohn et al, 1947) and forms a soluble complex which rapidly exchanges with the cellular membranes (Havel and Fredrickson, 1956; Laurell, 1956).

During short periods of exercise, the FFA of plasma have been found to increase (Carlson and Pernow, 1959; Friedberg et al, 1960; Carlson and Pernow, 1961; Bruce et al, 1961; Cobb et al, 1961). But it was reported by Carlson and Pernow (1961) that when the exercise was continued for longer periods, the FFA content increased to nearly the pre-exercise level. However, in the present study on the pigeon shows that there is a significant decrease in the plasma FFA level during 30 minutes of exercising the breast muscle. Few readings obtained after 1 houe of electrical stimulation of the breast muscles, also showed the same trend of decrease in the plasma FFA level.

The extraction of FFA from the blood plasma by the muscle during muscular exercise is indeed indicated in the present observations, by the fact of an increase of FFA in the muscle and its decrease in the blood. That the changes in the FFA levels were observed in the whole blood without studying the arterial and venous bloods separately was possible, is perhaps due to

the fact the bird muscle is a well adapted system for the metabolism of fat. In the case of the human system on the other hand, the capacity for fat metabolism is relatively low and hence the changes could be detected only by studying the difference in the arterial and venous bloods.

It is an established fact that the human myocardium extracts free fatty acids from the circulating blood and these fatty acids are the important metabolite of the heart (Bing, 1954; Gordon, 1957; Ballard et al, 1960). Bing (1954) and his associates have also suggested that if the extracted fatty acids are completely burnt, it could acdount for about 70% of the oxygen consumption of the heart. It has also been shown in the isolated rat heart preparation that the free fatty acids are preferentially oxidized when glucose and FFA are present in 5 mM and 0.4 mM concentrations respectively (Shipp et el, 1961).

It has been suggested by many workers that the FFA of the blood are utilized by the skeletal muscles during muscular activity (Fritz et al, 1958; Carlson and Pernow, 1959; Friedberg et al, 1960; Issekutz and Spitzer, 1960). Fritz and others have also shown that there is 60% increase in the oxygen consumption and a 100% enhanced oxidation of fatty acids during muscular exercise. George and Jyoti (1955; 1957) showed that there is a striking reduction in the fat content of the muscle and liver in the pigeon during the electrical stimulation of the muscle. Studies on the respiratory quotient after electrical stimulation of the muscles, have shown to be about 0.7 indicating that fat

is being burnt during muscular exercise (Pishawikar, 1961). From these observations and the data presented in this chapter, it could be concluded that the skeletal muscles of birds during sustained muscular activity utilize$ mainly fat for its energy. The fat store in the breast muscles of flying birds may be considered as an immediate reserve and as a special adaptation. In the muscles of non-flying birds the fat content is extremely low (Fowl for e.g. 0.98% in wet muscle) (George and Jyoti, 1955) and also the concentration of lipase (George and Scaria, 1956).

It is well known that the fat from the adipose tissue are transported to the organs needing fat in the form of FFA through the circulating blood. It has been calculated, that if transport of fatty acids out of depots as FFA is to account for the mobilization of the stored fat to supply the caloric need of the organism during fasting, a rate of FFA release to the order of 100 micromoles per gramme of tissue per hour would be necessary in rats of approximately 200 gm. weight (Gordon, 1958). It appears from the present studies and the studies reported by Shafrir et al, (1959) and Shafrir and Steinberg, (1960) that the first mechanism by which the organism meets its demands during stress reactions is by an increased mobilization of FFA by the adipose tissue. Therefore, the adipose tissue fat, sometimes considered to be a relatively non-labile store of substrate, appears under emergency conditions to be more rapidly available as a source of calories than carbohydrate (Shafrir and Steinberg, 1960). Certain preliminary studies conducted on the

total fatty acid content of the adipose tissue in the pigeons show a remarkable reduction after the breast muscle has been electrically stimulated for 30 minutes.

Hamosh and Shapiro (1961) have shown that liver injected with 1-C^{14} palmitate releases free fatty acids into the medium. The results reported in this chapter on the changes in the FFA content of the liver after 30 minutes of electrical stimulation of the breast muscles also give evidence to the fact that liver also lets free fatty acids into the blood stream under high energy demands.

The findings reported in this chapter could be interpreted to mean that FFA is the major link in the transport of fat from the fat depots and liver to the tissues and that the status of the energy metabolism in some way controls the FFA movement. It has been shown that epinephrine (Gordon, 1958) and nor-epinephrine (White and Engel, 1958) accelerate the release of FFA from the adipose tissue in vitro probably by accelerating the lipolytic enzymes(Shapiro, 1957; Wadstrom, 1956). Crag and Beetham (1957) have shown that the plasma concentrations of epinephrine and nor-epinephrine is increased during exercise. Thus the adipose tissue plays a central role in regulating the plasma FFA level and should be considered as a dynamic site regulating lipid metabolism.