SUBSTRATE UTILIZATION DURING EXERCISE

Muscular Efficiency When Fat is the Major Source of Energy

By

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ABSTRACT

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Previous investigators reported that muscular work efficiency decreases when fat is the major energy substrate. The decreased efficiency could result from dehydration caused by ketosis, or from glycogen depletion when high fat diets are fed. The purpose of this study was to investigate whether dehydration was a cause of impaired muscular work efficiency.

Dehydration was found to be related to protein rather than fat intake. A low protein diet caused dehydration; but this was not observed when a higher percent of protein was fed, regardless of the nature of the protein and fat. Two series of exercise experiments were performed, and despite a similar ketosuria, a decreased work efficiency was found only in the latter series, in which the carbohydrate intake was lower and the duration of the experimental periods longer. The more severe carbohydrate restriction presumably can cause glycogen depletion, which may be responsible for the decreased efficiency.

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INTRODUCTION

When Saul became king over Israel, he was involved in many wars with the Philistines. On one such occasion he forbade his men to taste anything while they were pursuing the enemy. His son Jonathan, with his armorer, did not hear the command and finding some honey in the field, they tasted thereof.

When Saul's men pointed out that they were forbidden to taste anything under oath, Jonathan replied that in this, Saul had commanded thoughtlessly because his men had become exhausted, whereas Jonathan and his armorer had become revived and their eyes were shining. (Holy Bible, I Samuel, chapter 14.)

This is probably the first recorded experiment on the effect of nutrition upon physical performance: It appears that a little bit of sugar was able to significantly increase the endurance--an observation that is very relevant today.

Krogh et al. (1920) studied the relative efficiency of fat and carbohydrates as a muscular energy source in man and found that fat was about 10% less efficient. Marsh and Murlin (1928) reported similar results. On this basis, it was generally believed that fat was less efficient than carbohydrate as an energy substrate for muscular work.

Christiansen and Hansen (1939) studied endurance on high fat and high carbohydrate diets in man; and found that endurance was less

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than half on a high fat diet as compared to a high carbohydrate diet. Bergström et al. (1967a) studied endurance on various diets and found, by taking needle biopsies from the exercising muscles, that the point of exhaustion correlated well with depletion of glycogen.

Since fat is the major substrate for endurance activities, it is unlikely that it is less efficient than carbohydrate for muscular work. It was therefore decided to investigate the possible role of ketosis and dehydration in decreasing the efficiency.

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REVIEW OF LITERATURE

PART ONE: SUBSTRATE UTILIZATION

I. EXERCISE AND SUBSTRATE UTILIZATION

When discussing the substrate used during exercise, it is very important to specify the type of exercise studied. Dynamic Muscular Endurance (DME) is the ability to perform under variable conditions, such as high intensity power exercise and endurance exercise.

The DME can be subdivided into nine physiologically different levels, each supposedly with a different pattern of substrate utilization (Van Huss, 1977). At the two extremes we have very short duration, high intensity exercise (60 yards dash) and endurance-type exercise such as the marathon.

A. Rest

In the resting postabsorptive state, fat has been shown to contribute the majority of the energy (Havel, 1971), but ketone bodies may also contribute to a limited amount (Ziegler et al., 1968). Glucose is also used to some extent and it is the only substrate for some tissues. In the postabsorptive state there is an increased release of amino acids (AA), particularly alanine, from the muscle; but local oxidation has not been proven (Felig and Wahren, 1970).

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B. Exercise of Short Duration and High Intensity

During this type of exercise the main substrate is preformed high energy phosphate; but some conversion of glycogen to lactate also appears to take place (Hultman, 1967). The oxygen consumption is very low. It is generally believed that the cytoplasmic ATP is compartmentalized, and that only a small fraction is in equilibrium with phosphocreatine (PC) (Bergström et al., 1971b). These authors also showed that the muscle level of PC is inversely related to the work load, whereas the ATP level is very little affected, except at very high intensity work. At exhaustive exercise, the PC level is reduced to less than 10%, whereas the ATP is reduced by about 40% of resting level.

C. Exercise of Short Duration and Low Intensity

Again, the phosphagens are an important source of energy; but it appears that glycolysis dominates as substrate, presumably because of the decreased rate of energy utilization, particularly as work time increases (Pernow and Wahren, 1962; and Jorfeldt, 1970).

D. Exercise of Long Duration and Low Intensity

As work time increases, the respiratory quotient (RQ) decreases from about 0.85 to about 0.70 (Havel et al., 1963). This indicates a shift to aerobic metabolism of free fatty acids (FFA). However, some muscle glycogen is still used, but this is primarily for aerobic oxidation (Hultman, 1967). As exercise continues, the fat supplies up to 90% of the substrate (nine hours work). At this time glycogen appears to be depleted, so the glucose is presumably supplied by gluconeogenesis.

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E. Exercise of Long Duration and High Intensity

When the intensity of exercise increases, the muscle begins to use substrate stored in the muscle cells. The glycogen content of the muscle falls rapidly, but lactate does not accumulate to any significant degree. It has been shown that duration of exercise under these conditions is well correlated with the initial glycogen content (Hultman, 1967).

The lipid stores in the cell are apparently also used under such conditions, but they do not become depleted, and the amount used or amount present before exercise is not related to the amount of work performed (Fröberg et al., 1971).

F. Summary

It is clear that the kind of substrate used for energy production depends very much upon the type of exercise performed. However, it is also influenced by the kind of muscles used for a particular task, which again, to some degree, is determined by the type of exercise performed. Muscles are generally classified as red oxidative and white glycolytic (Pette, 1971).

In evaluating the effect of a particular substrate, it is desirable to choose a type of exercise that primarily uses the substrate in question. At rest during the postabsorptive state, fat is the major substrate, as it is during exercise of long duration. During high intensity exercise at short duration, the phosphagens are the major substrates; but as the duration of the exercise increases, so does the importance of glycogen and blood glucose. During exercise of long duration and

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relatively high intensity, glycogen becomes progressively more important and may under such circumstances be a limiting factor.

II. FAT AND EXERCISE

A. Free Fatty Acids

1. Muscle Uptake. Fat is the major energy substrate for exercise of long duration, and it is available to the muscle cell in three forms: free fatty acids (FFA), triglycerides (TG), and ketones. Hagenfeld and Wahren (1968a) studied the metabolism in the human forearm during one hour of exercise. They found that the muscular extraction of FFA was proportional to the arterial concentration; however, since the arterial concentration increases during exercise, the uptake also increases. The fractional uptake of the FFA was about 15% (arterial concentration 440 to 520). They also found that the muscle has some preference for oleic and linoleic acid as compared to palmitic acid; this is in contrast to the exercising leg where Havel et al. (1964) did not find any difference. The decreased preference for palmitic acid has been confirmed by Nestel and Barter (1971), who found that the fractional turnover of linoleic acid was greater than palmitic acid, and that less incorporation in plasma triglycerides took place. The uptake of FFA could account for about 50% of the fat oxidation, and about 60% of the FFA was oxidized, the remainder going primarily to β-hydroxybutyrate. They also demonstrated that the exercising forearm was able to oxidize glycerol. However, recently Hagenfeld (1975) concluded that there is no significant difference in muscle uptake of the individual FFA, whereas the splanchnic uptake varies with each individual FFA.

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Hagenfeldt and Wahren (1972) found that at low work intensities, the uptake of FFA from the blood could account for all the fat oxidized, but at heavier work intensities, fat oxidation exceeded the uptake of FFA; and they suggested that fat stores in the muscles made up for the difference. They also found that during low work intensities, the fat was completely oxidized, whereas at higher intensities, only about 60-70% was oxidized to carbon dioxide, the rest of the label going to acetate, 3-hydroxybutyrate, 2-oxogluterate and citrate.

2. Splanchnic Uptake. When splanchnic FFA uptake was studied (Hagenfeldt and Wahren, 1973) by arterial and hepatic vein catheterization, arterial FFA, renal blood flow, and arterial - hepatic venous differences decreased during exercise, whereas lactate increased. Analysis showed that the decreased uptake is not solely due to lower arterial FFA level. They concluded that the splanchnic FFA uptake is reduced during exercise to allow greater FFA utilization by the muscle.

Hagenfeldt and Wahren (1975b) have recently shown that arachidonic acid is handled differently than oleic acid. The fractional uptake during rest in both splanchnic area and in muscles is 50% higher; furthermore, the turnover-rate is unaffected by exercise.

Jones et al. (1972) found that exercise under hypoxic conditions increased FFA metabolism as was the arterial (brachial) lactate concentration. Hagenfeldt and Wahren (1973) found that the hepatic arterial lactate level correlates negatively with the FFA level and hepatic extraction. Hagenfeldt and Wahren (1975a) have studied turnover of FFA during recovery from exercise by use of 14C labelled acid. The arterial concentration of FFA reached a maximum of twice the

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level during exercise six minutes after termination of exercise. They conclude that the postexercise peak is caused by release of FFA into the plasma pool; this in turn augments the removal of FFA.

Hagenfeldt (1975) has shown that the splanchnic fractional uptake of FFA increases as the chain length decreases and as the degree of unsaturation increases; and the uptake varies greatly with the individual FFA's. Hagenfeldt and Wahren (1973) concluded that the net splanchnic FFA uptake is reduced during exercise in order to permit redistribution of the turnover of FFA in the body, so that more FFA's are available for utilization by the muscle.

B. Triglycerides

Carlson et al. (1970) showed that plasma triglyceride (in humans) contributes to the energy supply of the heart. Recently (1975) they have investigated whether triglyceride also contributes to the energy supply used by working muscle in humans. The problem here is to measure the arterial-venous difference with sufficient accuracy. They were not able to detect any significant differences in concentration; but differences below the standard error would still be able to account for up to 25% of the energy utilization by the muscle. This could be at least part of the reason why the FFA extraction cannot account for all the fat oxidized during strenuous exercise.

Although fat appears to be the major substrate for muscle energy, feeding a high fat diet does not appear to increase performance. Krogh et al. (1920) found early that the muscular efficiency decreased 10-12% in subjects fed a high fat diet, a result that was confirmed by Marsh and

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Murlin (1928). Christensen and Hansen (1939) showed that the work time decreased by 2/3 when the subjects changed from a high carbohydrate diet to a high fat diet. Hultman and Bergström (1967) found similar results and they ascribed the decreased endurance to the depletion of glycogen stores. Henschel et al. (1954) found a decreased ability to perform hard work during acute starvation, and ascribed this to dehydration and acidosis.

C. Ketone Bodies

1. Purpose and site of ketone production. The discovery of ketone bodies in the urine resulted in the belief that they were associated with a pathological process or were products of abnormal metabolism. However, this view has given way to the physiological role of ketosis. Williamson (1971) suggests the following roles for ketones: They are important substitute fuels for the brain, and have an antilipolytic action. They can be considered normal fuels in situations such as starvation and exercise.

The principal site of ketone body formation is the liver (Wieland, 1968). The liver in turn, is unable to use ketones because it lacks 3-oxoacid CoA-transferase (Williamson et al., 1971). However, there is some evidence that β-hydroxybutyrate is produced by the muscle during exercise (Hagenfeldt and Wahren, 1968b), but it is probably less important than the liver production.

The ketone bodies can be considered an easily soluble transport form of energy, and they are particularly useful for the brain, where they at least partially can substitute for glucose as substrate (Owen et al.,

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(1967). Ketones have long been known to be the preferred fuel for the heart (Williamson and Krebs, 1961). After an overnight fast, the muscle consumption of ketones is low (in humans), but after three days of fasting with increased arterial concentration of ketones, the consumption increases dramatically. After twenty-four days of fasting, the arterial concentration of acetoacetate and 3-hydroxybutyrate is further increased; but the muscle utilization of these substrates remains unchanged or even decreases (Owen and Reichard, 1971b).

Ketosis appears to have some clinical significance in that the ketogenic diet forms the basis for an apparently fairly successful treatment of many types of epilepsy that are otherwise not easily controlled (Keith, 1963; and Livingston, 1973). It is generally believed that the formation of ketone bodies is the basis for the success of this dietary regimen.

It should be mentioned that besides the physiological ketosis and its clinical applications, there are cases of pathological ketosis in which ketone production appears to be out of control. Examples of this kind are the severe ketosis seen in the terminal stages of diabetes and that sometimes seen in lactating cows (Krebs, 1966). Pathological ketosis is always associated with excessive glucóneogenesis and Krebs (1966) suggests that the use of oxaloacetate for gluconeogenesis depresses the level of oxaloacetate to such a degree that the TCA cycle cannot handle the amount of acetyle CoA. However, during physiological ketosis a sufficient decrease of oxaloacetate has not been found.

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2. Mechanism of ketone production. Flatt (1972) found that gluconeogenesis has a permissive effect on ketogenesis. The link here is ATP. The conversion of fatty acid (FA) to ketones generates ATP, and unless the energy is used, the process comes to a standstill. Gluconeogenesis is an energy utilizing process; therefore, when ATP is used for gluconeogenesis, ketogenesis is allowed to proceed to a higher degree.

McGarry and Foster (1971a) investigated the ketogenesis by infusion of octanoic acid. This FA is not used directly for synthesis of triglycerides. With high concentrations of octanoate, the livers from fasted rats, unlike those from normal animals, could be induced to synthesize ketones at a rate approximately equal to diabetic rats. Perfusion studies with octanoate-1-14C revealed that the activity of the tricarboxylic acid cycle, although modestly decreased in the ketotic state, only moderately influenced the rate of ketogenesis. They also found that the rate of ketogenesis from octanoic acid is higher in the fasted state because of depressed lipogenesis: Normally this acid is elongated.

McGarry and Foster (1971a) similarly found that decreased TCA-cycle activity cannot account for the enhanced ketogenesis seen in fasting. They reported that the decreased triglyceride formation is sufficient to account for the increase in ketogenesis seen during fasting. One point generally agreed upon is that a raised plasma level of FFA is a necessary and important prerequisite for ketogenesis; but McGarry and Foster (1972) emphasize that an increased uptake of FFA by the liver in itself is not sufficient to initiate maximal ketogenesis.

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If decreased reesterification of FFA in the liver is responsible for ketogenesis, it can explain why medium chain triglycerides give a higher level of ketones than corn oil (Tantibhedhyankul et al., 1967; and Pi-Sunyer et al., 1969). Unsaturated fat on the other hand, gives lower levels of triglyceride in blood, despite faster incorporation of unsaturated FA into triglyceride. Hagenfeldt et al. (1972) have shown that the splanchnic fractional uptake is greater for unsaturated FFA than for saturated, and greater for short chain than for long chain FA, which is apparently due to faster esterification. Upon this basis, it is conceivable that the different FA should have different rates of ketogenesis.

The liver uptake of an individual FFA is proportional to its concentration in plasma (Hagenfeldt et al., 1972), so presumably there is control of ketogenesis at two levels: First, the plasma concentration is regulated, and secondly, there is regulation through the fate of FFA in the liver where the FFA can either be esterified, oxidized, or go to ketone bodies. As mentioned earlier, it has been suggested that if reesterification is depressed, as during the fasting state, the FA will be directed toward ketogenesis.

McGarry and Foster (1971a) have shown, however, that if the TCA cycle is blocked, reesterification occurs even in the fasted state, indicating that the esterification process is fundamentally intact. This means that there must be some other means of control.

3. Control of ketone production. The catecolamines, particularly norepinepherine, are important effectors of lipolysis (Rossel and Ballard,

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1971), and insulin is known to be a potent inhibitor of adipose tissue lipolysis (Cahill et al., 1966). Although controversial, it appears that glucagon also stimulates lipolysis in man (Gerich et al., 1976b). Bieberdorf et al. (1970), increased plasma FFA in fasting rats by infusing chylomicrons and heparin, but this did not increase ketone production. When they infused insulin, ketone production decreased in the presence of sustained high plasma FFA. Pi-Sunyer et al. (1970) found that hyper-ketonemia had a feedback effect on lipolysis in adipose tissue of dogs. Similar results were obtained by Bjørntorp and Schersten (1970). Madison et al. (1964) found that infusion of ketones in alloxan diabetic dogs caused hypoglycemia, decreased hepatic glucose output to 50% of control, and caused a greater than 50% fall in arterial FFA. They noted that these results are similar to those obtained by infusing insulin.

Balasse and Neef (1975) demonstrated that the ketone bodies have a negative feedback effect on their own production in humans. However, Senior and Loridan (1968) failed to find any increase in insulin level of man, although they found a significant decrease in FFA, glycerol and glucose upon infusion of 3-hydroxybutyrate. Grey et al. (1975) also found that insulin did not mediate the negative feedback of ketones upon lipolysis and ketogenesis in man, as it does in experimental animals.

4. Muscle metabolism. Hagenfeldt and Wahren (1968b) studied the metabolism of ketone bodies in human subjects during rest and exercise with 3-hydroxybutyrate-4-14C as tracer. During rest, they found that the uptake of ketones by the forearm muscle was proportional to the

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arterial concentration; but during exercise, there was no such correlation. On the contrary, they found a net release of 3-hydroxybutyrate, indicating intramuscular production of ketone bodies during exercise.

5. Post-exercise ketosis. The phenomena of post-exercise ketosis indicates the sharp raise in blood ketone level after the end of strenuous exercise. Johnson and Walton (1972) explain this phenomenon by the pronounced increase in FFA after the termination of exercise; under such condition (glycogen depletion), ketone production is proportional to plasma FFA. Rennie et al. (1973) found that post-exercise ketosis is less in trained athletes than in untrained, even when they work at the same relative workload.

Askew et al. (1975) studied this phenomenon in rats and found that training increased the capacity to oxidize ketone bodies; however, contrary to results in humans, they found higher blood ketone levels in trained than in untrained rats. This may be because the rats were exercised to complete exhaustion, whereas the results in humans were at submaximal workloads; or else it is possible that rats respond in a different manner than humans, in terms of ketone regulation during exercise.

D. Summary

Organs differ greatly in their handling of fat substrates. The muscle uptake is proportional to the arterial concentration, which increases during exercise. It appears that the fractional extraction of palmitic acid is slightly lower than for the other FFA's. In the splanchnic area, the FFA uptake decreases during exercise, and the fractional extraction decreases with an increase in chain length and the

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degree of saturation. Triglycerides are used as substrate by the heart, but this has not been proven to be the case in the muscle. Arachadonic acid is handled in a special manner by both the muscle and the splanchnic area. Its uptake is about 50% higher than other FFA's at rest, and is unaffected by exercise.

Ketones are primarily produced by the liver, and the production increases in situations where the supply of carbohydrate is limited. The ketones function as a substitute substrate for glucose, particularly in tissues (such as in the central nervous system) which are dependent upon glucose. The ketone production increases gradually, reaching a maximum the third or fourth day of glucose starvation; but because the utilization of ketones by the muscle decreases, the blood level of ketone bodies may increase until the seventh to tenth day of carbohydrate starvation. It appears that ketone production is regulated by a homeostatic mechanism. Though the regulation of ketone production is not completely understood, it is apparently regulated by the FFA concentration in the blood and the insulin/glucagon ratio. Both ketones and FFA increase in the blood after exercise, presumably due to increased lipolysis, and ketone production is affected by training.

III. CARBOHYDRATE METABOLISM

The regulation of blood glucose is one of the most important homeostatic mechanisms, and glucose can be used for energy substrate in most tissues, although some, such as the heart, seem to prefer FFA. However, other tissues such as the central nervous system, the renal medulla and the erythrocytes, normally use only glucose as a substrate

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Carbohydrate, besides being a direct source of energy, can be converted to fat and stored in the adipose tissue and, in small amounts, can be stored as glycogen (Hultman et al., 1974). While there is no evidence that fat can be converted to glucose, glycogen serves as an important source of glucose in maintaining the blood glucose level; the other important gluconeogenic substrate being protein (Weiss and Löffler, 1970).

Besides being oxidized to CO_2 and water, glucose can be metabolized to lactate (Hultman et al., 1974). The former process is far more efficient, but the latter has the advantage of being able to proceed under anaerobic conditions.

A. Lactate and Pyruvate

Cori and Cori (1928) described the cyclic process of glucose metabolism to lactate in the peripheral tissue (particularly muscle tissue). The lactate is then transported to the liver where it is resynthesized into glucose.

The lactate formation in skeletal muscle during exercise appears to be related to the relative work intensity; below 50% of aerobic capacity, little increase in blood lactate concentration is seen. At higher sub-maximal loads, blood lactate increases in proportion to the work intensity during the first minutes of exercise, and at close to maximal loads, a constant production is observed. Either lactate formation is related to hypoxia, or pyruvate is formed faster than it can be used and thus converted to lactate (Knuttgen, 1971). The lactate formed can go

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to the liver where it is resynthesized to glucose, or it can be used directly as an energy substrate in muscle tissue. It should be pointed out that not all the oxygen debt is due to lactate formation; the alactic debt appears to be due to the splitting of phosphagens.

Karlsson (1971a) has shown that there exists a considerable gradient for lactate between the intracellular space and the blood; the exact value depends upon the intensity of the exercise. This has been confirmed by Hirche et al. (1971) in rats. The work load applied was 50 to 60% of maximum oxygen uptake and the maximal lactate concentration in the muscle was close to 29 mmoles/liter wet muscle; the corresponding blood lactate concentration was about 15 mmoles lactate/liter blood. After extremely heavy bicycle exercise (exhaustion in 50 seconds), the maximal muscle lactate concentration was 39 mmoles/liter wet muscle. This gave a lactate ratio of up to six, between inter- and extracellular lactate. These experiments (Karlsson, 1971a) also indicate that the total lactate production at the time of exhaustion is constant, regardless of workload, time to exhaustion, or muscle lactate concentration at time of exhaustion. A good correlation was also found between blood lactate and muscle lactate concentration. Karlsson (1971a) found that pyruvate increases to only a minor degree, and suggests that oxygen deficit is involved in accumulation of lactate during heavy exercise. This may be mediated through the NAD/NADH ratio.

Saltin and Karlson (1971a) found that training significantly decreased the muscle lactate production, even when their subjects worked at the same relative oxygen consumption. One explanation for this may

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be that athletes use a larger proportion of the red muscle fibers. As the work load increases, more white fibers are activated, which can explain the increased production of lactate as the work load increases (Hermansen, 1971). The delay in adequate oxygen supply is often given as an explanation for lactate production. However, Carlson and Pernow (1961); and Keul et al. (1967) have shown that it occurs regardless of relatively high oxygen tension in the venous blood from the muscle, but this blood comes from both active and nonactive muscles. Hermansen (1971) finds that at exhaustive work below ten minutes duration, exhaustion occurs at about the same blood lactate level (18 mmoles/liter blood). He also shows that the rate of lactate production is closely related to the work intensity, and that there is a pronounced lactate production at work times as short as ten seconds.

Jordfeldt (1971) studied the turnover of ^{14}C-L(+)-lactate in human skeletal muscle during exercise and found a continuous uptake of lactate during exercise, most of which was oxidized to CO_2 and other metabolites. The fractional oxidation of lactate (% of lactate take up converted to CO_2) increased from 38% at ten minutes of exercise to 52% at forty minutes.

Hermansen et al. (1973) found that after a period of intermittent exercise, blood lactate decreased twice as fast when recovering at 65% of maximal oxygen uptake compared to receovery at rest. When exercising at 65% of maximal consumption, there was a slight increase in lactate at five minutes; but from then on, lactate levels decreased steadily. It is generally assumed (Rowell et al., 1966) that most of

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the lactate formed is removed by the liver; however, it is known that the resting skeletal muscle does use lactate (Jordfeldt, 1970), and the above results indicate an increased uptake of lactate by skeletal muscle during exercise.

Minaire and Forichon (1973) investigated the lactate metabolism and the lactate-glucose interconversion during prolonged physical exercise in dogs, by using ^{14}C-L-lactate and ^{14}C-D-glucose as tracers. They found that the rate of lactate formation increased (doubled) during exercise, and that almost 75% of the lactate produced during exercise was immediately oxidized in the muscle. The glucose turnover rate also about doubled, but there was no change in the fractional interconversion rates of lactate to glucose and glucose to lactate during exercise. Thus, despite increased lactate production, there was no accumulation because of oxidation in the muscle. They found that only 13% of the lactate removed was converted to glucose. It then appears that there is an intimate balance between lactate formation and its oxidation in the skeletal muscle during exercise. It is a question whether at low levels of exercise, lactate is not formed to any important degree, or whether it is oxidized to CO_2 as fast as it is formed in the skeletal muscle. However, it is clear that during exercise close to the maximal oxygen uptake, and at supermaximal oxygen uptakes, lactate accumulates in the blood (Hermansen, 1971).

It can be calculated that during endurance type exercise, the energy derived from lactate formation is insignificant (less than 0.5% of the extra energy cost of the exercise) (Minaire and Forichon, 1973).

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The question one must ask is: Why is the lactate formed at all? One bit of evidence for an attractive theory is the work of Hoffman (1973), indicating that there is a special membrane compartment of ATP which supplies energy for one component of the Na^+/K^+- pump. The source of this ATP is anaerobic glycolysis.

B. Glycogen

Although glycogen is found in most tissues, the only important ones in terms of quantity are the muscle and liver (Hultman et al., 1974). This does not preclude some functional importance of glycogen stored elsewhere, such as in the brain and nervous tissue (Janzo Koizumi, 1974).

The highest content of glycogen is found in the liver, where the mean value is about 50 g/kg under normal conditions. With a liver weight of 1.8 kg, the total amount of glycogen is about 100 g (Hultman and Nilsson, 1971).

The glycogen content of muscle varies depending upon the muscle in question, but in the quadricepts femoris muscle, Hultman (1967) found 14 g/kg. If we assume that the muscle mass constitutes 40% of body weight, a normal man will have a total of about 400 g of glycogen (in muscle and liver), but it varies with the metabolic condition of the subject.

1. Liver glycogen. Earlier (Cahill, 1964; and Samols and Holdsworth, 1968), it was believed that the rate of gluconeogenesis was sufficiently high to maintain the blood glucose levels, and that the liver glycogen stores were only used in emergency situations. However,

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it has now been shown that during starvation, there is a continuous decrease in the glycogen stores of the liver due to glycogenolysis. This is also the case on a carbohydrate-free diet (Nilsson and Hultman, 1973; and Nilsson et al., 1973). The rate of decrease in liver glycogen varied, but on the average it was 0.3 mmoles glycosyl units/kg liver/min. At such a rate the stores will be depleted after 24 hours of carbohydrate deprivation. Likewise, when the liver was depleted, or nearly depleted, by carbohydrate starvation or exercise, and a high carbohydrate diet was fed, the glycogen level increased to supernormal levels of about 500 mmoles glucosyl units/kg wet liver tissue (corresponding to 100 g/kg wet liver). When the subjects remained on a carbohydrate free diet, the glycogen level remained very low (Nilsson and Hultman, 1973).

During hard, dynamic exercise, the liver glycogen stores are utilized, and may be depleted in exercise of long duration. When glycogen is broken down, water is liberated. The relationship has been determined directly by muscle biopsy. When 1 gm of glycogen is used, 0.45 mEq. potassium and 2.7 g of water are released (Bergström and Hultman, 1972). If the glycogen is metabolized aerobically, an additional 0.6 g of water is formed. If an athlete uses 500 g of glycogen, he will liberate 1650 ml of water, which will give a weight loss of more than 2 kg.

It has been found that the glucose output from the liver increases from 0.85 mmoles glycosyl units/min at rest up to 6 mmoles glycosyl units/min at the end of a hard exercise period (Hultman, 1967). This increase is positively correlated both to work load and duration of

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exercise. In a study where the subjects performed heavy exercise after a period on a low carbohydrate diet and after a period on a normal mixed diet, the liver glucose production increased by a factor of three in both cases. After the mixed diet, the increase was almost entirely due to glucogenolysis, and the uptake of gluconeogenic substrate by the liver was unchanged despite a large increase in blood lactate. After the low carbohydrate diet, however, the liver glycogen was very low, and the uptake increased from 0.3 mmoles/min at rest to 2.14 mmoles/min during exercise, thus accounting for about 80% of the increase in the liver glucose output (Hultman and Nilsson, 1973).

Felig and Wahren (1974), on the other hand, have shown that the muscle output of alanine increases from 50% during mild exercise to 500% during severe exercise, and they estimate that the gluconeogenic AA contributes from 10 to 15% of the liver output of glucose.

The liver is the principal organ responsible for maintaining the blood glucose level, although, under conditions of metabolic acidosis, the kidneys play a part in gluconeogenesis in connection with ammoniagenesis (Steiner et al., 1968). In a resting man the glucose consumption is about 1 mol/day. The liver produces this glucose by either gluconeogenesis or glycogenolysis, and as mentioned earlier, the relative role of these two processes has been a matter of debate.

Nilsson and Hultman (1973) found in the postabsorptive state that glycogenolysis produced about 0.54 mmoles glucose/min, which means the glycogen stores will be depleted after 24 hours. Since the output by the liver is about 0.85 mmoles glucose/min, gluconeogenesis must

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supply about 0.31 mmoles glucose/min, which was experimentally verified by liver vein catheterization. After a low carbohydrate diet, the splanchnic glucose production decreased to 0.30 mmoles/min, but in this case there was no glycogenolysis since the liver was depleted of glycogen. This means that when the glycogen stores are depleted, there is not a compensatory increase in gluconeogenesis in the resting state, whereas during exercise there is a several-fold increase in liver glucose output. When liver glucose output falls during carbohydrate starvation, the systemic glucose utilization must necessarily decrease and it is only after a time lapse of at least 24 hours that ketosis begins to develop (Bloom, 1967; and Hultman and Nilsson, 1975). Williamson et al. (1971) found that the central nervous system (CNS) is able to use ketones already in the fed state, and it is probably the ketone concentration in the blood that determines its utilization. Oxidation of ketones in the CNS therefore appears to substitute for the decreased glucose utilization. If exercise is performed on a low carbohydrate diet, low blood sugar is occasionally seen (Bergström et al., 1967b)

The gluconeogenic substrates are: lactate, pyruvate, AA (in particular, alanine), and glycerol. Glycerol comes from hydrolysis of fat to FFA, and AA from protein degradation. Since lactate and pyruvate are formed by glycolysis, no new glucose is made by this process. Felig and Wahren (1974) have estimated the relative importance of the gluconeogenic precursors in the normal postabsorptive state: 10-15% from pyruvate and lactate; alanine, 5-10%; other AA, 5%; and glycerol, 3%. Glycogen normally contributes about 75%, but as fasting or glucose

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deprivation extends beyond 24 hours, this becomes practically zero, while gluconeogenesis apparently remains unchanged (Nilsson and Hultman, 1973).

a. Control of glucose production by the liver. Gluconeogenesis and glycogenolysis appear controlled mainly by hormones. There is an immediate effect involving changes in enzyme activity and substrate availability, and a long-term effect associated with de novo enzyme synthesis.

Epinepherine and glucagon stimulate glycogenolysis in the liver; this effect appears to be mediated through 3',5'-cyclic - AMP (Hultman et al., 1974). When gluconeogenic substrates such as alanine are infused under gluconeogenic conditions, the glucose production by the liver immediately increases. This indicates that substrate availability is limiting the glucose output (Hultman and Nilsson, 1975). Glucagon primarily increases the activity of the gluconeogenesis, while the glucocorticoids seem to stimulate de novo synthesis of gluconeogenic enzymes. Epinepherine and growth hormone appear to stimulate lipolysis. Insulin opposes all these effects.

2. Muscle glycogen.

a. Effect of diet. The glycogen content of quadriceps femoris muscle at rest has a mean of 85 mmoles glycosyl units/kg and varies from 60-120 mmoles/kg (Hultman, 1967). In the resting muscle no net glycogen consumption can be measured, and it appears to rely solely upon FA oxidation (Andrews et al., 1956). Complete starvation or a carbohydrate-free diet therefore has only minor influence upon the glycogen stores of the muscle, provided no exercise is performed.

A slight increase in muscle glycogen was observed when the subject changed from low to high carbohydrate diet. However, if the glycogen stores are first emptied by a period of exercise, a high carbohydrate diet will lead to rapid resynthesis of glycogen, whereas it will take up to nine days on a carbohydrate poor diet (Hultman and Bergström, 1967). If the muscle group is completely emptied of glycogen and the subjects are given a high carbohydrate diet, an overshoot in the glycogen content can be obtained, but only in the muscles that were depleted. On a high carbohydrate diet resynthesis of the glycogen stores is complete within 24 hours; however, the glycogen content continues to increase to very high values for up to eight days (Ahlborg et al., 1967a). If a low carbohydrate diet is given, resynthesis takes about ten days.

b. Effect of exercise. During muscle contraction, glycogenolysis and glycolysis increase in relation to the work load. The output seems to be regulated by the ATP concentration. During isometric contraction with maximal voluntary contraction force, only a fraction of the glycogen store is used; but during relatively heavy, dynamic exercise (about 85% of the subject's maximum oxygen uptake), the glycogen stores in the muscle are depleted and appear to be limiting for performance (Bergström et al., 1967b). At these work levels there is a linear relationship between work time (at a given level) and the size of the muscle glycogen stores prior to exercise. The glycogen stores in the muscle cannot be used to maintain the blood glucose level since the muscle does not contain glucose-6-phosphatase; it therefore serves strictly as a local substrate for energy production (Hultman et al., 1974).

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3. Glycogen in the central nervous system. Hultman et al. (1974) states that the brain contains practically no glycogen and only a very small amount is found in fat tissue and kidney; however, very small amounts may play functionally important roles and such seems to be the case in CNS and adipose tissue.

Shimizu and Kumamoto (1952), with the help of staining techniques, showed the presence of glycogen in neuropil-, neuroglial- and nerve cells in the brain of most mammalian species. They also showed that certain areas such as hypothalamus and area postrema, and area on the floor of the fourth ventricle, are particularly rich in glycogen. The gray matter always contains more glycogen than the white matter; glycogen is also found in some of the cell nuclei in the spinal cord.

According to Koizumi (1974), glycogen granules have been demonstrated with electronmicroscopy in nerve cell parikaryon, axons, axon terminals, and dendrites of specific regions in the CNS of various species. Shimizu and Kumamoto (1952) demonstrated the presence of glycogen in the cytoplasm of neuroglial cells, and suggest that it plays an important role in the mediation of nutrition from the blood to the nervous tissue proper.

Koizumi (1974) states that psychotropic drugs, especially CNS depressant drugs such as chlorpromazine, are responsible for an increase in brain glycogen, and this indicates either an inhibition of the glycolytic pathways or a depression of the functional activities in these areas.

Kumamoto (1953) studied the effect of starvation on brain and liver glycogen in rabbits. He found that brain glycogen decreased even

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faster than liver glycogen as fasting progressed, but the rate of decrease varied in the different areas of the brain. Vrba (1954) found that brain glycogen decreased with physical exertion (1.5 hours of swimming) in rats; and Jacoubek and Svorad (1959) found the lowest level of glycogen in rat brains after two to five hours of swimming.

Insulin decreases the level of glycogen and free sugar in brains of normal cats and, to an even greater degree, in animals made incapable of secreting epinepherine (no lipolysis, Kerr et al., 1937). Convulsive activity is accompanied by a decrease in brain glycogen, glucose, phosphocreatine, adenosine triphosphate lactate and inorganic phosphate. There is a tremendous increase in glucose metabolism during the abnormal neuronal activity associated with an epileptic attack (Klein and Olsen, 1947).

Ketone bodies or the associated acidosis are usually cited as the reason for the effectiveness of a ketogenic diet in treating epilepsy (Livingston, 1973). However, it seems reasonable to suggest that the depletion of brain glycogen stores, when the ketogenic diet is used, removes the energy source necessary for the tremendous neuronal activity associated with an epileptic attack.

The neuromuscular system is often implicated as a cause of fatigue. Astrand and Rodahl (1970) discuss whether fatique to voluntarily muscular effort is located in the CNS or in the neuromuscular junction, but in either case, this may be related to the decreased function of the Na^+/K^+- pump and the resulting ionic imbalance. Hoffman (1973) has shown some evidence for a specific membrane pool of ATP that is preferentially

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used by one component of the Na^+/K^+-ATPase. Parker and Hoffman (1967) have shown that ADP in this pool is phosphorylated only through anaerobic glycolysis in the red blood cells (RBC). If this is also the case in other cells, it can explain why glycogen is so important for nerve and muscle function, even though fat or ketones can supply the major part of the energy.

C. Summary

Carbohydrate is found in the body as blood glucose and glycogen. During anaerobic metabolism glucose is converted to lactate, and the amount of lactate produced depends upon the intensity of the exercise. The lactate production is influenced by training, and at exercise of high intensity and short duration, the total lactate production appears to be correlated to the time to exhaustion. The resting skeletal muscle does not metabolize lactate, but lactate becomes a substrate for the muscle during exercise. Lactate production is increased parallel with the activation of the white muscle fibers, but most of this lactate is immediately oxidized in the red muscle fibers, while the rest goes to the liver for gluconeogenesis.

The two principal sites of glycogen storage are the liver and the muscle, and the pattern of utilization varies in these two organs. The liver glycogen is used in support of the blood glucose level and will be depleted in about 24 hours during carbohydrate starvation. If carbohydrate starvation is followed by a high carbohydrate diet, the glycogen level increases above normal, but the liver glycogen is not repleted if a low carbohydrate diet is consumed. If exercise is performed when a low

carbohydrate or high carbohydrate diet is consumed, the liver glucose production will increase about three-fold in both cases; but in the former case, it is due to gluconeogenesis, and in the latter, to glycogenolysis. The liver production of glucose is regulated by hormones.

A low carbohydrate diet does not cause (or only slowly causes) a depletion of muscle glycogen, but it is depleted by exercise, and if followed by a high carbohydrate diet, supernormal glycogen levels are found. At work loads of about 85% of V_{O_2} max. the glycogen stores are correlated with the time to exhaustion. Glycogen stores are also found in the CNS, which are depleted on a low carbohydrate diet, and may be a cause of the fatigue usually experienced when consuming such diets (Bloom and Azar, 1963).

IV. PROTEIN METABOLISM

Since the days of Leibig, many workers have supported the notion that proteins are essential as energy substrates for work performance (Kraut and Lehman, 1948). However, it is now well-established that under most conditions, this is not the case. It is clear that when glycogen is present in sufficient supply, protein is not used for energy to any appreciable degree; but recently there has been much interest in the role of amino acids during work of long duration (that is, more than one hour) (Keul et al., 1972).

Consolazio et al. (1975) recently studied the effect of heavy physical activity and training for forty days on two levels of protein intakes (1.4 and 2.8 g/kg of body weight). Based on the high protein intake, it is not surprising that there was no difference in performance;

Nitrogen balances were positive. They found an increased loss of urea in the sweat, but surprisingly, no compensatory decrease in urinary nitrogen excretion on the high protein diet.

Rougier and Babin (1975) studied changes in blood and urine urea and uric acid in trained and untrained men during exercise of short duration (running 3.5 km on a treadmill at a rate of 14 km/hour at 5% elevation), and during long-lasting exercise (an average of 15 km) by running to exhaustion on the treadmill. During exercise of short duration, the blood urea increased 25% at the end of exercise and almost 50% one hour after exercise, but was back to normal the next morning. The pattern was similar during long-lasting exercise. Blood urea increased 40% at the end of exercise, about 55% after one hour, and was still 20% above normal the next day. The increase was higher in trained people. Similar changes were observed for uric acid. This increase in blood urea has been found by others (Refsum et al., 1973). Rougier and Babin (1975) tried to evaluate renal function immediately after exercise, one hour later, and the next day. They admit that their clearance values were not too reliable since the urine flow was often less than 2 ml/min. They found the greatest increase in urea excretion right after exercise of short duration associated with a temporary increase in the urine flow; one hour later, urea excretion and urine flow were below resting values. During the night and the next day, urine flow gradually increased, but urea excretion increased more than accounted for by the increased flow.

Uric acid excretion increased immediately after exercise and continued to be above normal until the next day. During long-lasting

exercise, urea excretion decreased due to decreased urine flow. But after the exercise, urea excretion continued to increase above resting values as urine flow continued to increase toward resting levels. They also measured the hematocrit and showed that the moderately increased hematocrit could account for only an insignificant part of the increase in the blood urea level.

The authors concluded that decreased urinary excretion could not account for the increase in blood urea, since some subjects maintained normal urine flow during exercise; furthermore, uric acid and urea were not retained to the same degree, and were not related to plasma creatinine levels. The authors did not measure sweat rates, but this has been studied by others (Cerny, 1973) who obtained similar (20% increase) results for serum urea and uric acid when their subjects exercised for two hours on an ergometer. The calculated sweat urea excretion was 20 times higher, and sweat uric acid excretion 10 times higher than during rest, whereas the kidney creatinine and urea elimination rate decreased by 40% and 50% respectively during exercise. They also concluded that the increase in blood urea after two hours is not due to decreased renal excretion, but to increased protein catabolism. Similar results have been found by Kchatorian (1972); and Porzolt et al. (1973).

It is clear that blood urea nitrogen is elevated after prolonged exercise and it does not seem to be due to dehydration or impaired renal excretion. It has been suggested that the amino acids are used for energy in the working muscle (Lowenstein, 1972). There is, however, no direct proof for this. But it is well-established that proteins play

a role in gluconeogenesis through the alanine cycle (Felig, 1975).

A. Effect of a Protein Diet

Tolstoi (1929) studied the effect of an exclusive meat diet on the blood constituents in two "normal" men for one year. Ketonuria was reported to occur daily, and blood uric acid concentration increased but returned to control levels after about three months on the meat diet. They had no control cholesterol values, but at the start of the meat diet, values as high as 800 mg/100 ml were reported, with an average of about 400 mg/100 ml.

McClellan and Du Bois (1930) report results from the same men on the meat diet (15-25% of the calories from protein, 75-85% from fat, and 1-2% from carbohydrate). The subjects lost an average of two kg during the first week on the meat diet, which the authors explain as a shift in the water content of the body, and nausea and weakness were reported in one subject. Increased urine volume was reported when shifting from normal to carbohydrate free diet, and it was lowest when carbohydrate was again added. Ketone bodies in the urine remained relatively high (up to 12 g/day). One subject was given a glucose tolerance test, and showed glucose uria. Urea clearance test showed above normal excretion of urea after one year on the meat diet; but after a period on mixed diets following the meat diet, the urea clearance was below normal. No elevation in blood urea was reported, but large variations were observed, probably associated with variations in protein intake. When the subjects switched to the high fat diet from the mixed diet, the blood ketone bodies increased until the fourth day, after which they remained constant in one

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Recently Heeley et al. (1975) studied the effect of a high protein diet in six young men for four days. The subjects lost an average of 1.4 kg during the four days, a significant increase in blood urea was found (from 4.65 to 8.13 mmoles/liter), and the urinary urea excretion increased from 23 to 39 g/day.

B. Protein Intake and Serum Urea

Addis et al. (1947) studied the relation between serum urea concentration and protein intake. The serum urea level was determined at three different protein intakes (0.5, 1.5 and 2.5 g/kg). They found considerable individual variation, but the percent increase in serum urea level correlated closely with the increase in protein intake; and the variation in blood urea level for each level of protein intake was about 15%. Taylor et al. (1974) showed that there is a correlation between serum urea and dietary nitrogen utilization; although, if the blood urea nitrogen is plotted as a function of protein intake, an equally good correlation is obtained, regardless of the source of proteins. However, when subjects were given 90-95% of their protein from wheat, their blood urea was only half that observed during an isonitrogenous control diet (Bolourchi et al., 1968). So it appears that under normal conditions, the serum urea level is proportional to the protein intake; but under certain conditions, other dietary factors appear to have an important influence.

C. Starvation and Muscle Protein Metabolism

During short periods of starvation, there is increased peripheral release of AA for gluconeogenesis (Pozefsky et al., 1976). The composition

and degree of weight loss was determined during different weight reducing regimens: An 800 kcal mixed diet, an 800 kcal ketogenic diet, and total starvation. The respective average weight losses were: 278 g/day, 467 g/day, and 751 g/day. The average protein losses were: 9.5, 17.9, and 50.4 g/day, respectively. And the corresponding water losses were: 101.7, 285.3, and 457.2 g/day. There was no significant increase in ketones on the mixed diet, but on the ketogenic diet and during starvation, the urinary excretion of ketones seemed to reach a plateau on the seventh day of about 2 and 7 g/day, respectively. There was a significent increase in urinary ketone excretion on the third day only (Yang and Van Itallie, 1976).

It is seen that the nitrogen excretion was only slightly higher on the ketogenic diet than on the mixed diet (same protein intake), but much higher during total starvation. Also, the subjects gained weight on the 1200 kcal post-experimental diet when it followed starvation or the 800 kcal ketogenic diet, whereas they continued to lose weight when it followed the 800 kcal mixed diet. This weight gain was due to water retention. They also found that the basic metabolic rate (BMR) was unaffected by the dietary regimen, although the volitional physical activity decreased 5% during and after the starvation period; and the energy cost of different activities was unaffected by the dietary regimen (as calculated from a record of the subjects' activity).

Yang and Van Itallie (1976) explain the increased weight loss as a shift in the water-balance during the different regimens. Looking at their data, however, it is clear that a substantial part of the weight

loss was due to glycogenolysis and protein catabolism (up to 500 g/day during the starvation period, including the associated water loss) (Olsson and Saltin, 1970). This interpretation is supported by the fact that the subjects gained weight on a 1200 kcal mixed diet when it followed the 800 kcal ketogenic diet, but not when following the 800 kcal mixed diet. It appears obvious that after the ketogenic diet the liver and probably some muscle glycogen is depleted, which is probably not the case after the mixed diet.

Blackburn and Flatt (1973) found that a daily protein intake of 0.6 to 1 g/day/kg of body weight (as the only food intake) was very similar to total fasting. The amount of urinary nitrogen is comparable, indicating that the rates of gluconeogenesis are similar. The ingestion of protein, however, will compensate for the degradation of AA and the nitrogen balance will be close to zero or even positive. The decrease in body mass is almost exclusively due to a temporary loss of water and decrease in body fat (Flatt and Blackburn, 1974). Infusion of AA without glucose does not prevent ketosis (Blackburn and Flatt, 1973), so fat is still mobilized to supply the energy need.

D. Effect of Fasting on Blood Uric Acid Level

Lennox (1924) studied the effect of total fasting on the blood uric acid concentration, and found that it increased. In seventeen subjects starved for eight days or longer, the uric acid rose on the average, from 4 to 11 mg/100 ml, an increase of about 165%. Uric acid began to increase on the third or fourth day of starvation, and was maximal after about a week, being maintained to the end of starvation.

This increase in uric acid appeared to be due to decreased excretion.

Lewis and Corley (1923) found similar results; the uric acid remained high when starvation was followed by a high fat diet. Harding (1927) observed that diets containing sufficient fat to produce ketosis also gave an increase in serum uric acid. Christofori and Duncan (1964) confirmed these results, and found that it was due to decreased AA and glucose reabsorption; these compounds normally compete with uric acid for reabsorption. Wilson et al. (1952) showed that a high protein diet increased uric acid excretion. Bonsnes and Dana (1946) suggested that there is competition between glucose and uric acid for reabsorption. Christofori and Duncan (1964) showed that glycine and glucose promptly increased uric acid excretion.

E. The Alanine Cycle

Felig and Wahren (1974) postulated a glucose-alanine cycle involving peripheral synthesis of alanine by transamination of pyruvate. Protein metabolism is important for glucose homeostasis, since AA are released by the muscle and gut, and extracted by the liver. Alanine, which is rapidly converted to glucose in the liver, constitutes a large part of the interorgan flux, despite the fact that it accounts for less than 10% of the AA in the muscle. Glutamine also appears to play an important role. One important aspect of the alanine cycle is that it removes ammonia from the muscle in a nontoxic form.

1. The amino acid exchange in the postabsorptive state. The observations on the net balances of AA across muscle, liver, gastrointestinal tract, kidney, and brain in normal man in the postabsorptive state

clearly demonstrate the key role of alanine and glutamine in the overall flux of AA between tissues (Felig, 1975). Free AA are released from muscle and gut, and are extracted by the liver. Alanine and glutamine account for more than 50% of the total alpha amino nitrogen released by the muscle. The gut also releases substantial amounts of alpha amino nitrogen, primarily as alanine. The liver is the major site of alanine uptake, where its extraction exceeds that of all other AA. Glutamine is primarily taken up by the kidney and gut, and used for alanine synthesis in the gut (Matsutaka et al., 1973) and for ammoniagenesis in the kidney (Cahill and Owen, 1970). The branched chain AA (valine, leucine, and isoleucine) are taken up by the brain rather than the liver (Felig et al., 1973).

2. Alanine and gluconeogenesis. Since alanine is the primary AA released by the muscle, it must be the most important AA for gluconeogenesis, and indeed, gluconeogenesis has been demonstrated to increase proportionally to the availability of alanine (Hultman et al., 1974). For most AA, gluconeogenesis is saturated at three times their normal plasma AA concentration, but alanine does not reach saturation until about 25 times normal concentration. ^{14}C-labelled alanine is promptly incorporated into glucose in the postabsorptive state and after prolonged fasting (Felig et al., 1970a). While all AA other than leucine are potentially gluconeogenic (Krebs, 1964), alanine accounts for more than half of the AA used for hepatic gluconeogenesis. In the postabsorptive state, glycogenolysis accounts for about 75% of glucose formation, and alanine accounts for about 30% of the remainder (Felig, 1973). It is therefore mainly in

situations where glycogen is depleted, such as prolonged fasting, ketotic hypoglycemia and long-lasting exercise, that the alanine cycle can achieve functional importance.

3. The glucose-alanine cycle. Alanine accounts for more than 30% of the alpha amino nitrogen leaving the muscle, but muscle protein only contains 7-10% alanine (Odessey et al., 1975). Alanine must therefore be synthesized de novo in the muscle by transamination of pyruvate (Felig et al., 1970b). In the liver, alanine is then converted to glucose and urea. The amino-group, however, must come from somewhere, and Felig and Wahren (1971a) suggest that the branched chain AA supply the amino group. Muscle has been demonstrated to be the site of oxidation of the branched chain AA (Manchester, 1965). There appears to be a linear relationship between pyruvate and alanine, which is not observed for any other AA, and ^{14}C-labelled glucose indicates that 60% of the alanine released is derived from exogeneous glucose degradation products (Odessey et al., 1975). It is noteworthy that the branched chain AA account for only 10-22% of the alpha AA released from the forearm muscle (Felig et al., 1970b), although these AA constitute about 20% of the muscle proteins (Odessey et al., 1975). It thus appears that muscle proteins not only participate in glucose homeostatis, but are also metabolized locally for energy. Also, physiological increments of branched chain AA increase the formation of alanine from ^{14}C-labelled glucose (Odessey et al., 1975), and the release of amino groups from the branched chain AA can account for all the nitrogen recovered in alanine. Other AA added to the plasma fail to increase the alanine output. It has been suggested that the branched chain AA are released from

the liver to complement the glucose-alanine cycle (Odessey et al., 1975). Since lysine is not synthesized or catabolized in the muscle, its release is a measure of muscle proteolysis, and since the muscle alanine content is similar to the lysine content, it can be estimated that 67% of the alanine released from the muscle is derived from glucose degradation products (Felig, 1975). This has been confirmed with labelled glucose in rat diaphragm (Odessey et al., 1975). Felig (1975) also calculates that the alanine cycle operates at a rate of about 50% of the Cory cycle.

4. Purine nucleotide cycle. Besides functioning in glucose homeostasis, the alanine cycle also transports amino groups to the liver in a nontoxic form. The branched chain AA are preferentially oxidized by the muscle tissue, and this may be particularly important during exercise, since ammonia is produced by the contrasting muscle (Lowenstein, 1972). The ammonia production appears to be proportional to the amount of work. Lowenstein and Tornheim (1971) have proposed a purine-nucleotide cycle in which ammonia is released from AMP by the adenylate diaminase, and suggest that the ammonia has a regulatory effect on the energy-supply in muscle tissue. Tornheim and Lowenstein (1973) have demonstrated that the purine-nucleotide cycle and glycolysis are closely linked. The glucose-alanine cycle may also be important in terms of energy production. Conversion of glucose to alanine provides 8 mmoles of ATP compared to 2 mmoles ATP for lactate formation (Odessey et al., 1975). If one considers the oxidation of branched chain AA facilitated by alanine formation, 30-40 mmoles of ATP are formed per mol of AA oxidized (Krebs, 1964).

5. Protein feeding. When a protein meal is ingested, the plasma level of branched chain AA increases, while that of alanine decreases, regardless of the composition of protein (Frame, 1958; and Armstrand and Stave, 1973). Animal studies show that following protein ingestion, alanine predominates in portal flow, while glutamate and aspartate are absent. Uptake of alanine by the liver exceeds the gut output, indicating continual peripheral release in the absorptive state. Hepatic uptake of branched chain AA is low. The production of alanine by gut and muscle thus appears to take place in the absorptive--as well as postabsorptive state (Felig, 1975). There is evidence that this is also the case in man (Frame, 1958). Elwyn (1970) has suggested that plasma protein synthesized in the liver undergoes hydrolysis in the peripheral tissue, and thereby participates in the inter-organ AA transport. It must be remembered that the erythrocyte participates in the inter-organ transport of AA (Felig et al., 1973).

6. Starvation and the alanine cycle. Starvation can be divided into an early and a late phase. In the early phase, the response is primarily directed toward maintaining glucose homeostasis; but in the latter, toward preserving the muscle protein stores (Felig et al., 1969). Hultman and Nilsson (1971) have shown that the glycogen stores are rapidly depleted during fasting. Felig et al. (1969) state that there initially is an augmented hepatic uptake of glucose precursors, particularly alanine, to maintain blood glucose production. This appears to be different from feeding a carbohydrate poor diet, where Nilsson et al. (1973) found no change in the hepatic uptake of alanine when the liver

is depleted of glycogen. The cause of this difference is not known but on the carbohydrate diet, the caloric supply is adequate; during starvation, it obviously is not.

The increased gluconeogenesis seen during the first three days of starvation is due both to increased hepatic extraction of alanine (Felig et al., 1969) and to increased release of alanine from the muscle (Pozefsky et al., 1974). These changes in alanine metabolism appear to be related by the insulin/glucagon ratio. As starvation continues for weeks, there is a gradual decrease in protein catabolism as evidenced by a fall in the urinary nitrogen excretion (Owen et al., 1969). The decreased gluconeogenesis is made possible because the increased ketone production replaces glucose as an energy substrate in the brain (Owen et al., 1967). In this second phase of starvation, a general decrease in the plasma AA concentration is seen, and the alanine concentration in particular, decreases (Felig et al., 1969) because of decreased muscle output of alanine (Felig et al., 1970b). The hepatic alanine extraction is increased, if anything, and if alanine is given orally or intravenously, a prompt increase in hepatic glucose output is seen (Felig, 1972).

The hepatic uptake of alanine is controlled by the insulin/glucagon ratio, and catecolamines may also play a role (Mallette et al., 1969). Glucagon has no effect on muscle release of alanine, and insulin has very little effect (Rudeman and Berger, 1974). Felig (1975) suggests that blood ketone level may have a feedback effect on muscle alanine release; however, on a low carbohydrate diet, alanine appears to continue being released despite elevated ketone bodies (Hultman and Nilsson,

1975). In this situation, though, the alanine output was at all times much lower.

The branched chain AA are rate-limiting essential precursors for protein synthesis, and in addition, they (especially leucine) promote synthesis and decrease degradation of proteins directly (Buse and Reid, 1975; and Fulks et al., 1975). When the carbocyclic analogs of the branched chain AA were fed to fasting man, a significant reduction was seen in nitrogen excretion (Sapir et al., 1974). As starvation progresses, ketone bodies increase in the blood, mainly due to decreased metabolism of the keto acids in the muscle (Owen and Reichard, 1971a). Garber et al. (1974) has shown that ketoacid production by the splanchnic bed is maximal by the third day of starvation. Fatty acids become the preferred substrate (Cahill, 1976), the oxidation of the branched chain AA decreases (Aoki et al., 1975), and blood levels increase. The increase in branched chain AA limits alanine output (Buse and Reid, 1975; and Fulks et al., 1975). The protein sparing effect of the AA is used clinically in starvation (Blackburn et al., 1973; and Hoover et al., 1975).

7. Diabetes mellitus and the alanine cycle. The diabetic environment is somewhat similar to that of short term starvation, and probably even more comparable to that of a high fat diet. In short term starvation, there clearly is a caloric deficit; but in both diabetes mellitus and a high fat dietary regimen (less than 6% of calories from carbohydrates), there is an adequate caloric supply, but a functional or real shortage of glucose. In both short-term starvation and diabetes mellitus,

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there is an increased branched chain AA concentration in the plasma, whereas alanine and other glycogenic AA decrease. Splanchnic uptake of gluconeogenic precursors (lactate, pyruvate, and alanine) increases 50-100% so that the precursors account for about 40% of the hepatic glucose output, compared to 15-20% in the normal postabsorptive state. This augmented glucose production is the result of a 2-3 fold increase in hepatic fractional extraction of the glucose precursors (Wahren et al., 1972).

8. Exercise and the alanine cycle. The effect of exercise on muscle AA metabolism varies with the duration and intensity of the exercise. During brief exercise (10-40 minutes), the only AA to have an increase in arterial concentration is alanine, and an output of this AA is observed from the exercising limb. The increased alanine output varies from 50-500%, depending upon the intensity of the exercise. The arterial alanine concentration increases 25-100%, and is directly proportional to the arterial pyruvate concentration (Felig and Wahren, 1971a). Stimulation of branched chain AA metabolism has been demonstrated during exercise (Turner and Manchester, 1973); and so has conversion of aspartate to oxaloacetate (Randle et al., 1971). This presumably partly accounts for the ammonia source for the increased alanine production. The alanine cycle thus provides a nontoxic mechanism for removing ammonia from the muscle. The splanchnic extraction of alanine during brief exercise is similar to the basal state; but since the hepatic glucose output increases three- to four-fold, its relative contribution to the glucose production decreases markedly.

During prolonged exercise (four hours), splanchnic release as well as hepatic extraction of alanine increases. As exercise continues beyond 40 minutes, splanchnic alanine extraction increases while peripheral release of alanine continues at elevated levels, resulting in a decreased arterial concentration of this AA. The overall uptake of glucose precursor increases about three-fold, so that gluconeogenesis accounts for about 45% of the hepatic glucose output. This situation is similar to that observed in diabetic patients (Wahren et al., 1972), and during short term fasting (Felig et al., 1972). The decreased insulin/glucagon ratio is probably responsible for the increase in gluconeogenesis (Ahlborg et al., 1974).

During prolonged exercise, the branched chain AA become important as fuel for the exercising muscle (Ahlborg et al., 1974). A consistant uptake by the exercising leg is demonstrable for valine, leucine, and isoleucine; and there is a close relation between splanchnic production and muscle utilization of these AA during exercise. Glucagon appears to stimulate the release of branched chain AA from the liver (Mallette et al., 1969), while glucagon, epinephrine, and FA stimulate the oxidation of these AA by the muscle (Buse et al., 1973). During recovery, splanchnic extraction of gluconeogenic precursors increases 45-100% as a consequence of increased fractional extraction, and the total precursor consumption accounts for about 45% of the hepatic glucose output. Uptake of alanine and release of glucose remain elevated during the recovery phase (Wahren et al., 1973).

F. Summary

When sufficient glycogen is present, proteins play only a minor role as energy substrate; however, when the glycogen stores are depleted (or nearly depleted), proteins appear to play a functionally important role as gluconeogenic substrate. The blood urea nitrogen (BUN) increases during exercise, and does not appear to be caused by decreased urinary excretion, but by protein catabolism. The BUN increases when the protein intake increases, but it also increases when a low carbohydrate diet is consumed, partly due to protein catabolism, or, to a direct affect of this dietary regimen on the kidney.

The two primary AA released from the muscle are alanine and glutamine. Glutamine is converted to alanine in the gut, and alanine is extracted by the liver where it accounts for more than 50% of the total AA uptake. Glutamine is also used for ammoniagenesis in the kidney. The branched chain AA are primarily used by the muscle and in the brain. In the muscle, the branched chain AA are either used directly or transaminated to pyruvate to form alanine. The gluconeogenesis from alanine in the liver is controlled by the insulin/glucagon ratio, but the release of alanine from the muscle appears to be controlled by the AA profile in the blood (increased level of branched chain AA).

V. HORMONAL CONTROL OF SUBSTRATE UTILIZATION

When man progresses from the absorptive state to the postabsorptive state, a change in substrate flux takes place that appears to be controlled by hormones. As fasting progresses, hormones further regulate the substrate availability. During the absorptive state the

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hormonal environment favors storage of substrate, while in the postabsorptive state, we see substrate mobilization (Owen and Reichard, 1971a).

A. The Insulin/Glucagon Ratio

1. Glucose homeostasis. The primary effect of the insulin/glucagon ratio is to maintain glucose homeostasis. A high ratio favors the uptake of glucose by muscle tissue (liver and central nervous system not affected), and the conversion of glucose to fat in the liver. Similarly, when arterial glucose concentration falls, the insulin/glucagon ratio decreases, which promotes gluconeogenesis in the liver from the appropriate substrates (Unger and Orci, 1976).

2. Insulin/glucagon ratio and AA. AA stimulates the release of both insulin and glucose (Pagliara et al., 1974; and Gerich et al., 1974a), but in general they are much better stimulators of glucagon secretion. The individual AA differ in their ability to affect the insulin/glucagon ratio. In man, arginine, lysine, and leucine are the most potent insulin stimulators (Fajans and Floyd, 1972), while arginine, glycine and alanine are most effective for glucagon secretion (Müller et al., 1975; and Wise et al., 1973). The branched chain AA (leucine, isoleucine and valine) have no effect on glucagon release (Rocha et al., 1972).

In vitro studies indicate that AA stimulate insulin release somewhat, but are potent stimulators of glucagon; however, in man the presence of glucose augments the insulin response to AA (Levin et al., 1971) while decreasing the glucagon response (Pagliare et al., 1974). AA, in conjunction with glucose, therefore play an important role in

regulating the insulin/glucagon ratio. The release of glucagon appears to prevent hypoglycemia otherwise caused by aminogenic insulin secretion (Unger et al., 1969).

Insulin stimulates protein synthesis (Wool et al., 1972). This is partly due to increased transport of AA into the cell (Cahill et al., 1972b), but insulin also decreases AA catabolism (Fulks et al., 1975).

3. Insulin/glucagon ratio and FFA, ketone bodies. During glucose starvation, hepatic glucose production increases. Initially, glycogenolysis dominates; however, when glycogen is depleted, gluconeogenesis (primarily from AA) becomes the only source of hepatic glucose production. During prolonged starvation, the continued loss of protein is incompatible with life (Cahill et al., 1966). After several days of starvation, gluconeogenesis from AA decreases while ketone production increases and replaces glucose as the energy substrate for the central nervous system (Cahill et al., 1966). McGarry et al. (1975) suggest that appropriate changes in the insulin/glucagon ratio are partly responsible for ketogenesis. The mechanism whereby glucagon induces ketogenesis has not been elucidated, but it appears that a low level of glycogen and a high hepatic carnitine level are necessary. The hepatic ketogenic capacity does not increase until an hour after administration of glucagon; but unless insulin is simultaneously reduced, there is not sufficient lipolysis to sustain ketogenesis.

In vitro FA and ketone bodies stimulate insulin release in the presence of non-stimulatory glucose concentrations (Hawkins et al., 1971). These agents also inhibit glucagon secretion in vitro (Edward

and Taylor, 1970). Ketone bodies stimulate insulin secretion in some species such as dog and rat (Madison et al., 1964; Hawkins et al., 1971; and Balasse et al., 1967), but in man and other species, this does not seem to be the case (Balasse and Ooms, 1968). Acute elevation of FFA has little effect on the plasma insulin level in fasting man (Balasse and Ooms, 1973; and Pelkowen et al., 1968); it does, however, increase the insulin response to subsequent glucose stimulation (Balasse and Ooms, 1973). Similar experiments show that lipids supress glucagon secretion (Andrews et al., 1975; and Gerich et al., 1974b); this action, unlike that of glucose, does not require insulin.

It has been proposed that plasma FFA and ketones during starvation release enough insulin to prevent keto acidosis (Seyffert and Madison, 1967); and the inhibition of glucagon could be important, since it supposedly would decrease lipolysis and decreases ketone production by the liver (Gerich et al., 1964b). However, in man, FFA and ketones do not appear to influence the insulin/glucagon ratio (Balasse and Ooms, 1973).

Insulin is known to be a potent inhibitor of lipolysis (Cahill et al., 1966). Glucagon on the other hand, is a powerful lipolytic agent (Steinberg et al., 1959), but there appear to be important species variation. Gerich et al. (1976b) recently showed that glucagon under certain circumstances can induce lipolysis in man; but others have not found lipolytic action (Marliss et al., 1970; and Lefebvre, 1972). However, glucagon is known to stimulate insulin secretion (Samols et al., 1966), which will counteract the lipolytic effect.

4. Effect of insulin/glucagon ratio on gluconeogenic capacity. It is well established that insulin lowers the AA content in the plasma of humans (Felig et al., 1969a; and Zinnerman et al., 1966). The decrease is most pronounced for the branched chain AA, methionine, tyrosine, and phenylalanine; and the effect is due to inhibition of AA output from the muscle (Pozefsky et al., 1969), except for glutamate for which an uptake has been demonstrated (Aoki et al., 1973). Thus, the result is a decrease in plasma AA.

In contrast, alanine concentration in the blood is not lowered by insulin, nor is muscle output inhibited; but when endogenous insulin secretion is stimulated by glucose, increased arterial alanine levels are observed in normal man (Felig et al., 1975). However, under appropriate conditions, insulin stimulates incorporation of alanine into muscle protein (Manchester and Young, 1970). The seemingly anomalous behavior of alanine can be explained by the insulin-induced synthesis from pyruvate in the so-called alanine cycle (Felig et al., 1970b; and Felig, 1973).

Insulin increases utilization of glucose by fat and muscle tissue, and inhibits its release from the liver (Madison, 1969). Studies of splanchnic AA balance after intravenous and oral glucose administration (to stimulate insulin secretion) indicate that the decreased gluconeogenesis is due to an effect of insulin on the liver (Felig et al., 1975; and Felig and Wahren, 1971b).

Insulin decreases alanine release from the muscle, since the arterial concentration of this AA is unchanged, or even increased, and

^{14}C-alanine incorporation into glucose in the perfused rat liver is inhibited (Rudorff et al., 1970). Since some alanine continues to be taken up by the liver, it must be disposed of in a different way during hyperinsulinemia.

The older studies of glucagon were performed with pharmacological levels rather than with physiological levels, which are about 100-200 pg/ml (Unger, 1974). It is difficult to interpret studies with pharmacological levels of glucagon, since they result in insulin secretion (Samols et al., 1965).

Physiological increments of glucagon during prolonged fasting resulted in about a 15% decrease in plasma AA; but there was no increase in urea excretion. So under these circumstances, there does not appear to be any catabolic or gluconeogenic effect (Marliss et al., 1970). Infusion of glucagon in high physiologic amounts has no effect on AA balance in the forearm in either the postabsorptive state or during short-term starvation (Pozefsky et al., 1974). Physiological levels of glucagon in arterial blood do not increase incorporation of alanine into glucose (Chiasson et al., 1974).

B. Growth Hormone

Evidence for the participation of growth hormone in human lipid and carbohydrate metabolism is based largely on experiments with pharmacological quantities of growth hormone, or with experiments in hypophysectomized individuals. In pharmacological doses, growth hormone decreases glucose utilization (Felig et al., 1971; and Fineberg and Merimee, 1974); it also induces lipolysis and ketosis (Raben and Hollenberg, 1959; and Felig et al., 1971). Using physiological levels

however, similar results have not been reported (Gerich et al., 1976b). Hypophysectomy decreases ketosis and lipolysis as well as insulin requirements in diabetics (Pearson et al., 1960), but these effects may not be solely due to growth hormone (Harlan et al., 1963), and mobilization of FFA and ketosis occur despite growth hormone deficiency (Merimee et al., 1971). However, Gerich et al., (1976b) recently showed that under some conditions, physiological levels of growth hormone do augment lipolysis and ketonemia in man; but in the presence of physiological levels of insulin, these actions are normally not apparent.

During exercise after an overnight fast, human subjects showed an increase in blood levels of growth hormone (Hunter et al., 1968). It is not known what activates this rise in growth hormone, but it has been suggested that it serves a protein preserving effect, and that it may be triggered by a change in the plasma AA profile; it is also observed in the long-term fasting state (Hunter, 1972). Alanine presumably could play such a role, and Becker et al. (1975) investigated the effect of alanine in protein calorie malnutrition in children, assuming that the low alanine seen in these patients caused the high levels of plasma growth hormone. They were surprised at seeing an increase instead of a drop, but this result fits the interpretation that alanine causes growth hormone secretion.

C. Thyroid Hormone

Triiodothyronine (T_3) enhances catabolism of protein stores, which is indicated by a doubling of urea excretion during a fast when T_3 was given, compared to the control period (Carter et al., 1975). Creatinine excretion increased six-to nine-fold, and increased plasma

levels of FFA as well as ketones were seen. The urinary excretion of ketones also increased. However, hyperthyroidism may simply increase the energy requirement.

It has recently been shown that there is both an active and inactive form of triiodothyronine, and although the thyroxine level remains normal during starvation, the active triiodothyronine (3,5,3-T_3) decreases strikingly, whereas the inactive triiodo (3',5',3-T_3) increases (Portnoy et al., 1974; and Vagenakis et al., 1975). When refed a high carbohydrate diet, man increases the active triiodothyronine and decreases the inactive triiodothyronine.

D. Glucocorticoids

Glucocorticoids exert their effect over a period of time, probably through de novo synthesis of gluconeogenic enzymes (Hultman et al., 1974). Animal studies show a protein catabolic effect in the muscle, and release of AA for gluconeogenesis in the liver when fasting animals are treated with glucocorticoids (Smith and Long, 1967). Similar data is not available for man; but hyperadrenocorticism shows increased plasma alanine, while other AA are unchanged (Wise et al., 1973). Hydrocortisone increased plasma alanine without change in other AA in experimental animals (Betheil et al., 1965). Corticosteroids also increased glucagon secretion (Marco et al., 1973), suggesting that the increased gluconeogenesis may be indirect, through the action of glucagon. After prolonged starvation, glucagon fails to increase protein catabolism (Owen and Cahill, 1973), but this may be due to elevated growth hormone levels.

E. Hormone Regulation in Exercise

In evaluating the role of the various hormones during physical exercise, one must consider whether the work is of short duration or continuous, severe or mild, and whether the subjects are trained or untrained, normal or abnormal. The subjects' ages and sex are also of importance. Furthermore, prior nutritional history can influence such factors as glycogen stores, which may effect the hormonal response.

1. Insulin/glucagon ratio during exercise. Insulin inhibits both hepatic glycogenolysis and gluconeogenesis, but there appears to be a difference in the sensitivity of these two reactions toward insulin (Felig and Wahren, 1975). Glycogenolysis appears to be more sensitive than gluconeogenesis to small increments of insulin (Felig and Wahren, 1975). This has also been shown with high and low insulin doses during infusion of labelled alanine (Liljenquist et al., 1974).

Serum insulin decreases during long as well as relatively short-term exercise (Wahren et al., 1971; and Ahlborg et al., 1974). Such a decrease in insulin is known to increase hepatic glucose output (Felig and Wahren, 1971b; and Wahren et al., 1971), and it will favor lipolysis (Cahill et al., 1966). During heavy work and very prolonged exercise, plasma glucagon concentration also increases, and may contribute to the increased fuel delivery (Ahlborg et al., 1971; and Felig et al., 1972). The insulin and glucagon responses to strenuous exercise are believed to be mediated by the sympathetic nervous system, and adrenergic blocking agents greatly reduce the exercise-enduced hyperglucagonemia (Harvey et al., 1974; and Luyckx and Lefebvre, 1972). However, recent evidence

suggests that changes in the insulin/glucagon ratio are not the sole determinant of hepatic glucose production during exercise. If plasma insulin and glucagon are maintained at basal levels by glucose infusion, hepatic glucose output still increases two to three times during exercise (Felig et al., 1974a). When hyperinsulinemia (more than 100 micra units/ml) is maintained by infusion of insulin, exercise still stimulates hepatic glucose output (Felig and Wahren, 1975). It is suggested that catecholamines which are released under these circumstances, influence the hepatic glucose output during exercise.

It is generally accepted that insulin secretion is blocked by catecholamines during exercise (Pruett, 1970); for that reason, during exercise of long duration, the enhanced glucose utilization must be attributed to a factor(s) other than insulin (Oseid and Hermansen, 1971). The glucose response to work of long duration depends upon both the intensity and duration of the work. Wahren et al., (1971) suggested that the blood insulin level decreases in order to preserve glucose for the brain; however, it is evident that glucose uptake in the muscle is stimulated despite decreased insulin levels (Metivier, 1973). During short-term, intermitant exercise, Hermansen et al. (1970) found that both plasma immuno reactive insulin (IRI) and blood glucose increased (four-fold and two-fold, respectively). Similar increases were seen during glucose infusion, so under such conditions (bouts of maximal exercise), insulin secretion is not inhibited.

Hansen (1971) compared the response of diabetics to nondiabetics during relatively short exercise (20 minutes). The diabetics all showed

an immediate, pronounced increase in growth hormone levels in the blood, but there was no change in the nondiabetic controls. During long-term exercise, Ericksson et al. (1971) found a significant increase in plasma growth hormone. Hartog et al. (1967) found no change in growth hormone until after 20 minutes; it then rose rapidly until 60 minutes, afterwhich it fell progressively. In these cases, growth hormone seemed not to be triggered by blood lactate plasma FFA or the plasma level of glucose. Buckler (1972) has shown that growth hormone secretion during exercise appears to depend upon cumulative effects. Hansen (1970) infers that there is a humoral factor released from the working muscle that triggers growth hormone release. Alanine presumably could serve such a role, and it deserves to be further investigated.

Schalch (1967) suggests that growth hormone has an insulin-like effect (short-term exercise) and a lipolytic effect (long-term exercise). While the latter has been shown (Gerich et al., 1976b), there appears to be no direct evidence of the former, although there is much suggestive evidence.

There is strong evidence to suggest that the corticosteroids play an important role in the energy supply during long-term exercise (Hazar et al., 1971). Others, however, have found a decrease of 17-hydroxycorticosterone during exercise (Viru and Oks, 1972), which may be due to distribution of the hormone in a larger tissue volume (Cornil, 1965). Metivier et al. (1973) found an increased secretion of growth hormone as work intensity increased; but though the results on cortisol are difficult to interpret, cortisol appears to be important during heavy exercise.

2. Aldostrone, cortisol and plasma renin activity. During prolonged, heavy exercise, a somewhat variable increase in plasma renin activity (PRA) is seen (Bozovic et al., 1967), but during exercise of shorter duration, the rise in PRA is related to the work load as well as to plasma norepinephrine (Kotchen et al., 1971). Since it is well known that exercise decreases renal blood flow (Castenfors, 1967; and Grimby, 1965), it appears that exercise causes increased sympathetic nerve activity, leading to decreased renal blood flow, which in turn stimulates renin release.

Sundsfjord et al. (1973) found that long-lasting exercise (4.5 to 7 hours) increased aldosterone (26 to 134 pg/ml), PRA (0.20 to 3.30 ng aug. I/ml/h), and cortisol (12.6 to 34.9 g/100 ml); but they found a relatively poor correlation among these variables. Aldosterone secretion is controlled both by the renin system and ACTH. Since cortisol increased, ACTH must also have increased. Because angiotensin II inhibits cortisol production, cortisol could not have increased without stimulation from ACTH (Rayyis and Horton, 1971). Hepatic blood flow is decreased during exercise (Rowell et al., 1964); and since aldosterone is primarily cleared by the liver, plasma aldosterone will increase because of decreased removal by the liver (hepatic extraction of aldosterone is almost complete). This means that there are different factors acting simultaneously on the aldosterone level which can explain the great individual variation in this parameter. As serum potassium levels increased only slightly (from 4.38 to 4.71 meq/L), the direct stimulatory effect of serum potassium does not appear to be responsible for the increase in serum aldosterone levels.

The ^{125}I-o-iodohippurate clearance decreased from 538 g plasma/min during rest to 343 g plasma/min during exercise (2.5 hours at 60% maximal oxygen uptake), and aldosterone clearance also decreased. However, equilibrium (isotope tracer) was not obtained during exercise. Tracer studies showed that duration of exercise as well as work load influences the cortisol level. A decrease in the latter was seen during the first hour, which may indicate increased distribution or metabolism followed by increased secretion, or decreased metabolic clearance rate on continued exercise. Sundsfjord et al. (1973) have shown that during the first 60-90 minutes, the renin-angiotensin system is the primary stimulator of aldosterone (Metyldopa, a blocker of renin release reduced both PRA and PA during this time); but after this period, aldosterone does not increase further despite continued increase in PRA and ACTH, and decreased metabolic clearance. After about 90 minutes, it appears that the adrenal is unable to increase its aldosterone secretion; the secretion may even decrease.

F. Hormone Regulation During Starvation or Carbohydrate Restriction

During starvation or carbohydrate restriction, normal individuals show increased glucagon levels (50 to 100% above the postabsorptive concentration) (Müller et al., 1971), and a decreased insulin level. Thus, the insulin/glucagon ratio (molar) falls to about 0.4, the same as seen during severe exercise (Unger, 1971). This ratio maximizes the hepatic glucose production, although during short-term starvation, glucocorticoids also appear to be important for gluconeogenesis (Felig, 1975), and growth hormone seems to limit it during long-term starvation

(Hunter, 1972). Unger and Orci (1976) state that the low insulin/glucagon ratio initially promotes glycogenolysis and when the glycogen stores are depleted, enhances gluconeogenesis and eventually ketone production. However, Nilsson et al. (1973) found that there was no increase in gluconeogenosis on a carbohydrate free diet, despite depletion of glycogen, and that gluconeogenesis was the same as in the postabsorptive state. Presumably this can be a difference between total starvation and carbohydrate starvation.

G. Summary

While glucose stimulates insulin, AA primarily stimulate glucagon secretion; however, the presence of glucose decreases the effect of AA on glucagon while increasing the stimulatory effect of AA upon insulin secretion. Insulin promotes glucose utilization and stimulates protein synthesis, whereas glucagon promotes hepatic glucose output. Physiologically, it is the insulin/glucagon ratio that is important. A decreased insulin/glucagon ratio is a necessary factor in ketone production. Physiological levels of FFA and ketones do not appear to have any effect on the insulin/glucagon ratio in man. Glucagon alone, stimulates lipolysis in man; but it also stimulates insulin secretion, which counteracts this effect. Insulin inhibits lipolysis. Glucagon does not stimulate the output of most AA from the muscle.

When the insulin level is low, growth hormone increases lipolysis and ketosis in man. Growth hormone secretion increases during prolonged starvation and exercise of long duration. T_3 enhances protein catabolism, but does not increase during starvation; however, there appears to

be a shift from the active to the inactive form, which is also seen on a high fat diet. Upon refeeding a high carbohydrate diet, this is reversed. Glucocorticoids also increase gluconeogenesis from proteins, but the effect may be indirect through secretion of glucagon.

During exercise, serum insulin concentration decreases, which increases hepatic glucose output, first from glycogenolysis (most sensitive) and then, from gluconeogenesis; during heavy and prolonged exercise, serum glucagon also increases. Catecolamines released during exercise appear to have a direct stimulatory effect on the glucose output from the liver. Growth hormone is released during exercise, and besides a lipolytic action, it seems to have an insulin-like action. There are changes in the aldosterone secretion during exercise, but the significance of this does not seem clear. During both long-term and short-term starvation as well as severe exercise, the insulin/glucagon ratio falls to 0.4, which maximizes the hepatic glucose production.

PART TWO: EFFECTS OF LOW CARBOHYDRATE DIETS

VI. EFFICIENCY AND HIGH FAT DIET

A. Substrate Efficiency

Zuntz (1911) concluded that the working muscle can use equally well the major sources of energy (fat and carbohydrate). From the studies of Fletcher and Hopkins (1971), Krogh et al. (1920) concluded that carbohydrate should be more efficient if the theories of Fletcher and Hopkins were correct, since according to their study, fat must first be transformed to compounds closely related to carbohydrates. For this reason, Krogh et al. (1920) decided to test the results of Zuntz (1911).

The subjects were fed either a high fat diet or a high carbohydrate diet. The composition of the diets is not given, but the major foods eaten are listed, and from them it can be concluded that they were diets very high in fat and carbohydrate, respectively. The subjects were fed each diet for four days; and they performed uncontrolled exercise during the first two days on the high fat diet, in order to deplete their glycogen stores. Two subjects started with the high carbohydrate diet and two with the high fat diet, in order to minimize the training effect. Both diets were low in protein.

The work was performed for two hours on the bicycle ergometer in the postabsorptive state. Results were collected only for the last 1.5 hours. Before exercise started, the resting oxygen consumption was

determined for ten minutes, after twenty minutes of complete rest (sitting in a chair). The efficiency was calculated as the workload divided by the oxygen consumption during exercise minus the consumption during rest. The oxygen consumption was converted to the caloric equivalent based on the RQ.

Their results showed that the muscular efficiency was about 10% higher on the high carbohydrate than on the high fat diet. They also found an increasing efficiency with training; and interestingly, they found (by comparing results during different times of the same exercise period) that the energy requirement increased as fatigue approached. That is, at exhaustion the efficiency was less than in the non-exhausted condition. This effect of fatigue showed itself much earlier when the high fat diet was fed (low RQ) than when the high carbohydrate diet was fed. The resting metabolism also depends upon diet, being lowest on a mixed diet, and increasing about 5% on a high fat diet and about 3% on a high carbohydrate diet.

Both the net energy expenditure per unit of work and the ratio of fat to carbohydrate catabolized are linear functions of the RQ; so regardless of the amount of fat oxidized, Krogh et al. (1920) concluded that 11% of the calories supplied by fat was wasted.

When work starts, the respiratory quotient changes. If it is close to one, it decreases, and if it is very low, it increases. On this basis, it was suggested that when the RQ was below 0.8, carbohydrate was formed from fat; and when the RQ was above 0.9, carbohydrate was converted to fat.

Marsh and Murlin (1928) made a similar study of the efficiency for one subject fed a high fat diet. The compositions of their diets were: high carbohydrate diet had 8% of calories from fat, 80% from carbohydrate, and 12% from protein; and the high fat diet had 80% from fat, 12% from carbohydrate, and 8% from protein. The high fat diet provided a slightly higher caloric content (2800 kcal/day compared to 2400 kcal/day).

In these experiments, as in those of Krogh et al. (1920), a bicycle ergometer was used for the exercise, but the work period was only eight minutes compared to two hours in Krogh's experiments, and it was done twice a day during the seven to eleven days each experimental period lasted. First they measured the efficiency when the normal diet was consumed, then followed a high carbohydrate dietary regimen and a high fat dietary period. The high carbohydrate and high fat period was repeated. They collected urine and calculated the nonprotein RQ. Furthermore, the urine was collected for one hour immediately before exercise (pre-work); and the urine collected during the work period included the recovery period and extended into the post-work resting period.

The average efficiencies were: 22.1% for the normal diet, 22.7% for the high carbohydrate diet, and 21.5% for the high fat diet. During the high fat dietary regimen, there was a progressive decrease in efficiency from the third or fourth day until the end of the period. This was not seen during the high carbohydrate dietary regimen. Extended to pure carbohydrate, they found fat to be 11-12% less efficient than carbohydrate.

They also found that the urinary nitrogen excretion decreased from the pre-work to the work period, with about 50-150 mg/hr; but not as much as when the high fat diet was consumed (about 100 mg/hr less). They concluded that this was due to a protein sparing effect of the glycogen mobilized during exercise. Less glycogen is available when a high fat diet is consumed (Nilsson et al., 1973). The urine excretion of nitrogen is also decreased during exercise (Rougier and Babin, 1975), whereas sweat excretion increases (Cerny, 1973), making an interpretation difficult.

B. Endurance

Christensen and Hansen (1939) compared the endurance during a high fat diet, normal diet, and a high carbohydrate diet. The composition of these diets were: high carbohydrate, 4107 kcal--protein 5%, fat 3%, and carbohydrate 83%; high fat diet, 5448 kcal--protein 2%, fat 95%, and carbohydrate 3%. These data are calculated from the diets listed for two specific days, and may not necessarily be the average (not given).

As Krogh et al. (1920) had found, work decreased the high RQ, but increased the low RQ. The RQ decreased as work progressed, particularly on the high carbohydrate diet. Substrate utilization based on the RQ (not corrected for protein) showed that in the beginning, carbohydrate contributed about 75% of the energy, but the proportion contributed by fat increased to 70% at the end of exercise (that is, point of exhaustion). Christensen and Hansen (1939) also found a gradual increase in the oxygen uptake which became much steeper as the point of exhaustion

was approached; this was associated with a fall in blood glucose level. Calculation based on the RQ showed that at the time of exhaustion on the high carbohydrate diet, about 400 g of glucose had been used for energy compared to about 50 g when the high fat diet was consumed. During the high fat diet, fat contributed up to 90% of the substrate, and the endurance was only about 1/3 of that seen during a high carbohydrate diet; there was a significant drop in blood glucose level at the time of exhaustion.

They found that the oxygen uptake per minute fell some at very high RQ values, but otherwise, it was constant above a RQ of 0.80. When the RQ was between 0.80 and 0.74, there was a very steep increase in the oxygen utilization per minute. The oxygen uptake increased 10 to 15% as the RQ fell from 0.8 to 0.75. It must be pointed out that if the increase in oxygen consumption is due to lowered efficiency of fat as substrate, and if the RQ accurately reflects the kind of substrate used, one would expect to see a straight line relationship between the RQ and the oxygen uptake; but experimentally, it is more like an all or none type response. That is, as glycogen is depleted, the oxygen uptake/min increases or the efficiency decreases. Finally, it should be mentioned that the oxygen consumption was not related to ketonuria; that it was just as high during periods of low ketonuria as when ketonuria was at a high level.

In subjects fed a single meal of fat and protein, Goldsmith et al. (1971) found a 10% increase in the oxygen consumption compared to the oxygen consumption in the postabsorptive state the same day;

whereas no increase was found after a carbohydrate meal.

C. Starvation and Work Performance

Henchel et al. (1954) studied the ability to perform hard work during acute starvation. During a 2.5-day fast and a 5-day fast, they found a weight loss of 4.5 kg and 5.5 kg, respectively. The work was walking on a treadmill at a 10% grade at 3.5 mph for four hours/day and three hours/day, respectively. There was no deterioration in work capacity the first day; but during work on the second day, blood glucose fell about 20% and the heart rate increased about 10%. The mechanical efficiency decreased about 6% during the 2.5-day fast. There was a definite increase in blood lactate and a 10-minute oxygen debt in the 5-day fast. Fitness generally deteriorated as the fast progressed. An increase in urine and blood acetone concentration in connection with the weight loss is taken to indicate that the acidoses causes dehydration; which in turn causes a loss in efficiency. However, the weight loss during the 5-day fast was not much higher than during the 2.5-day fast, despite the fact that blood ketones are known to increase progressively until the end of the second week of starvation (Cahill et al., 1966). Furthermore, a weight loss of this order can be partially explained by depletion of glucogen stores (Olsson and Saltin, 1970).

The effects of consecutive fasting (five to six weeks apart) have been studied by Taylor et al. (1945). The subjects maintained their blood sugar levels better during work in the fifth period (second and third days of fasting) than in the first period. The urine acetone and the urinary nitrogen excretion were higher during the fifth fast

compared to the first period of fasting; however, the increased urinary nitrogen excretion was not consistent in all subjects, so increased gluconeogenesis is not the sole factor in the adaptation to fasting.

D. Possible Explanation for Decreased Efficiency

From the previous results, it is seen that the oxygen consumption is indeed increased when fat is the major substrate, but it does not appear due to a decreased deficiency from using fat as a substrate, since the efficiency does not decrease until the carbohydrate intake reaches a certain low level; and the work of Christensen and Hansen (1939) does not show any relation to ketosis. Hultman and Bergström (1967) indicate that the decreased efficiency is due to glucogen depletion.

Krogh et al. (1920) suggest that the changes in RQ from rest to exercise are due to conversion of fat to glucose during a high fat dietary regimen. There is no evidence for such a conversion, but gluconeogenesis from protein probably does occur as well as glycogenolysis, which can alter the RQ. On the high carbohydrate diet, the decrease in the RQ during exercise is ascribed to lipogenesis, which presumably can occur, since on a high carbohydrate diet a large fraction of the glucose is converted to fat. Such interconversions, if they do occur during exercise, could significantly alter the energy requirement for a given task (Milligan, 1971). Using animal models, the in vivo energy yield from different substrates such as glucose and fat, has been shown to be proportional to their calorimetric values, indicating that the efficiency of utilization is equal, or, that the oxygen consumption of

fat or carbohydrate should correspond to the theoretically predicted consumption (Milligan, 1971).

When the relation between energy intake and energy retention was studied in animals, both below and above maintenance levels, fat was found to be used more efficiently, resulting in a smaller increment of heat than does carbohydrate. Protein was used much less efficiently than was carbohydrate, this conclusion was true irrespective of species or feeding level. At below maintenance level, the energy must be entirely used to support body function, and therefore must be related to the efficiency of the particular substrate used. When the efficiency found calorimetrically was plotted against the efficiency calculated biochemically relative to glucose, a very good agreement was found except for ketone bodies, acetic acid, and butyric acid. The explanation given for this is that as a basis for the calculations, constant turnover was assumed; and that is not valid when butyric- and acetic acids are given. The animals developed acidosis and ketosis, blood sugar fell precipitously, and urinary nitrogen excretion increased. As a small amount of propionic acid prevented these changes, it seems likely that gluconeogenesis from proteins was increased, and resulted in the lower efficiency (Blaxter, 1971).

There appears to be a relationship between the percentage of fat, carbohydrate and protein in the diet, and the efficiency of energy utilization in experimental animals. At low and high intakes, the ratio of fat to carbohydrate plays a role in the energy utilization. At high protein levels, a high fat/carbohydrate ratio increases the energy

utilization; but at low protein levels, a high fat/carbohydrate ratio depresses energy utilization. At intermediate protein levels, there was no effect of the fat/carbohydrate ratio (Hartsook and Hershberger, 1971). A similar relationship, if it holds in man, may be important, since most of the studies of efficiency have been done at low protein intakes.

E. Summary

There is not sufficient evidence to support the idea that fat is used less efficiently than carbohydrate as substrate; but the decreased efficiency seen on a high fat diet is probably due to other factors. One explanation given above is that it is due to gluconeogenesis, but other explanations will be considered.

It is well established that a high fat diet can increase the oxygen consumption, and decrease the time to exhaustion. It has also been shown that work performance deteriorates during starvation. A high fat diet and starvation both cause increased blood levels of ketones and a depletion of the body stores of glycogen, and may cause dehydration. The decreased efficiency generally ascribed to fat as substrate may therefore be due to a variety of factors, such as dehydration, acidosis, glycogen depletion, gluconeogenesis from protein, or a combination of these.

VII. EFFECT OF DEHYDRATION ON EXERCISE

It has been suggested that ketosis produces dehydration, and that this is the reason for the poor exercise performance during

starvation (Henschel et al., 1954). Pitts et al. (1944) studied the effect of different dehydration levels during exercise on a treadmill. The subjects received water ad lib, water consumption equivalent to sweat loss, or no water during exercise. The performance as evaluated by rectal temperature was best when the water intake corresponded to replacement of the sweat loss. Apparently the subjects did not voluntarily consume enough water.

In studies with restricted caloric intake but adequate water consumption, maximal strength was not affected until the weight loss reached about 10% in both acute and chronic starvation (Keys et al., 1950; and Taylor et al., 1945). Bosco et al (1974) found a loss in endurance when dehydration was caused by starvation as well as by water deprivation, but there was also a significant decrease in performance of the control group; so other factors such as motivation must be involved.

Saltin (1964b) studied dehydration caused by heat exposure, exercise, and both. Dehydration was continued until the subjects reached the same weight loss by each method. The performance was compared before and after dehydration at different work loads. Under no circumstances was the oxygen uptake (of the same workload) affected by dehydration, but at the lower workloads, the heart rate increased (mean of 13 beats/min). However, at maximal load, there was no change in heart rate, but the time to exhaustion decreased markedly.

Kozlowski and Saltin (1964b) studied the sweat loss and water distribution during thermal dehydration and exercise dehydration.

TBW (tritium space), ECF (insulin space), and PV (Evans blue) were determined before and after dehydration; sweat loss was also determined. The average weight loss was 4.1% or 3.1 kg, and the decrease in ECV was 1.4 and 0.2 liters respectively. The decrease in plasma volume was 0.7 and 0.1 liters respectively. There was no significant difference in the electrolyte loss, which was almost entirely eliminated through sweat. Na^+ loss was higher during dehydration, whereas K^+ loss was higher during exercise. There was no significant difference in the composition of sweat and urine during the two types of dehydration. The small decrease in extracellular fluid during exercise was surprising; however, this has also been found in prolonged heavy exercise (Astrand and Saltin, 1964). The energy use during dehydration and exercise was 500 (for the time required to loose the desired weight) and 3,100 calories respectively (the calory consumption was calculated from the respiratory quotient). In the case of exercise, this corresponds to an endogenous water production of 1.1 liters, but despite this water, there was a higher intracellular water loss during exercise dehydration. The increase in potassium in the plasma after exercise was not due to increased protein catabolism, but it might be due to glycogenolysis (Saltin, 1964a,b). It was found that a given sweat loss due to exercise dehydration is more detrimental to physical work capacity than the same sweat loss due to thermal dehydration; and exercise dehydration is primarily intracellular as compared to thermal dehydration, which is primarily extracellular (Saltin, 1964a and 1964b; and Astrand and Saltin, 1964).

Costill and Saltin (1974) found that during dehydration, the RBC shrink and thus, the venous hematocrit and the change in PV is underestimated. Costill et al. (1974a) found that the percentage change in mean corpuscular volume (MCV) is accurately described by changes in the mean corpuscular hemoglobin concentration (MCHC). Taking change in the MCV into account by measuring MCHC, Costill et al. (1974a) found that during onset of exercise, PV decreased 12.2% (due to transcapillary fluid flux in the muscle); but during the final 110 minutes, PV only decreased an additional 3.6%. Thermal dehydration resulted in only a 3% decrease during the first 10 minutes of heat exposure, but a 15% decrease during the remaining period. The difference may be due to degradation of glycogen. During onset of exercise, muscle water increased 9%, but this disappeared after two hours of exercise. During the first 30 minutes of recovery after thermal dehydration, PV increased 7%, and the rectal temperature decreased. This suggests that during thermal dehydration, water enters the dermal tissue because of peripheral vasodilation and sweating, and then re-enters plasma during recovery. The decrease in PV was about the same in thermal and exercise dehydration in contrast to the results of Kozlowski and Saltin (1964). Serum protein concentration reflects changes in PV following thermal dehydration, but not after exercise dehydration (Costill and Sparks, 1973). Costill and Saltin (1973) concluded that the decreased performance during exercise dehydration compared to thermal dehydration is due to glycogen depletion.

That ionic changes occur in the contracting muscle was shown by Hodgkin and Horowitz (1959). During exercise of relatively short

duration, potassium is lost from the muscle (Ahlborg et al., 1967b), but apparently not after about one hour of prolonged exercise (Haralambie, 1973a). An increase of extracellular fluid in the muscle has also been observed (Ahlborg et al., 1967b), which may explain the increased plasma protein and hematocrit seen during exercise (Bergström et al., 1971). The change in muscle potassium has been suggested as a limiting factor in exercise (Streter, 1963). Bergström and Hultman (1966) found a correlation between potassium and the glycogen content: 0.5 mEq potassium accumulates for each gram of glycogen deposited. This potassium is liberated from the muscle when glycogen is broken down (Bergström et al., 1967). Olsson and Saltin (1970) found an increase in total body water of about 2.5 kg during a high carbohydrate dietary regimen following work. Bergström et al. (1971a) concluded that the water and electrolyte changes following exercise are not of such magnitude as to limit exercise.

The changes in intracellular water and electrolytes seen during exercise can be explained largely by an accumulation of lactate and other metabolites in the muscle fibers (Karlsson, 1971a). There is a good correlation between lactic acid accumulation and the increase in intracellular water seen during exercise (Bergström et al., 1971a). As glycogen is depleted during long-lasting exercise, and fat supplies most of the energy, the lactate gradient probably disappears and this can explain the redistribution of PV seen in the beginning of exercise, but not later on during exercise (Costill and Fink, 1974). It appears that there is some difference between thermal- and exercise dehydration;

the latter gives a much more severe impairment of endurance, probably due to depletion of glycogen. Water loss as such, unless severe, does not particularly restrict exercise.

Summary. A sweat loss of about 10% is detrimental to physical performance, but it makes a difference whether this sweat loss is obtained through exercise or thermal dehydration. Performance is most affected by exercise dehydration, and this is probably caused by a decrease in the glycogen stores. The performance decrease seen during starvation, may also be due to depletion of glycogen stores. It has been suggested that the ketosis of starvation or a high fat diet causes dehydration through a loss of sodium in the urine.

VIII. WATER BALANCE AND CARBOHYDRATE RESTRICTION

A. Weight Loss

When obese patients were put alternately on 1,000 calories high fat, high carbohydrate, and high protein diets, they lost weight much more rapidly on the fat and protein diets than on the mixed diet, and they maintained weight for a few days while consuming the 1,000 calorie high carbohydrate diet when it followed the high fat or high protein reducing diet. The absorption of fat was above 90%, eliminating fecal loss as the explanation. The nitrogen balance was about the same on the high fat and high carbohydrate diets (that is, slightly negative on the average). During the high protein regimen, a definitely positive balance was observed at all times. The obese subjects showed resistance to hypoglycemia, severe ketonemia and acidosis while consuming the high

fat, low caloric diet, compared to nonobese subjects. The insensible water loss was higher during the high fat and high protein diets than during the high carbohydrate diet. Kekwick and Pawan (1957) explain their results as due to differences in metabolism between obese and non-obese subjects.

Benoit et al. (1965) compared the effect of starvation to a 1,000 calorie ketogenic diet and a 1,000 calorie mixed diet as control (half the subjects fasted first, and half consumed the ketogenic diet first). The mean weight loss during the 10-day periods was 9.6 kg, and 6.6 kg for the fast and ketogenic periods respectively. They found the negative nitrogen balance during the ketogenic period comparable to that of the mixed diet; and both much less (three times) than during total starvation. They also found the increase in plasma and urinary ketone bodies to be greater during the ketogenic diet than during starvation; and a negative potassium balance was seen only during the starvation period. A calculation of the composition of the weight loss for fasting gave 64.6% lean body tissue and 35.4% fat; and for the ketogenic, only 3% lean body tissue.

The main point of Kekwick and Pawan's study (1957) is that the body water/body weight ratio remained unchanged during the different dietary periods, and that the difference in weight loss therefore, was due to metabolic alterations. However, a look at their water-balance data clearly indicates that their accuracy was not good enough to detect a shift in water-balance (Olesen and Quaade, 1960).

B. Reason for Weight Loss

Olesen and Quaade (1960) studied the weight loss in obese women fed a 2,000 calories (25 g CHO) high fat diet, and found a remarkably high weight loss during the first 5-7 days; whereupon, the weight became stabilized for the remainder of the period (13 days). During the last 5-7 days when all the patients were fed a 1,250 calorie high carbohydrate diet, the weight remained constant even though caloric intake was below their measured basal metabolic rates. The constancy of weight must be due to water retention and possibly glycogen storage. The authors concluded that the subjects must have been dehydrated during the high fat diet, as indicated by the initially rapid weight loss. The only patient who was able to consume the 2,600 calories high fat diet (as used by Kekwick and Pawan, 1956) initially lost weight, but after one week, gained.

Pilkington et al. (1960) repeated the experiments of Kekwick and Pawan (1956), but for longer periods (24 days), and found no difference in the weight loss in the subjects when fed a 1,000 calorie high fat or high carbohydrate diet. However, body weight increased when the subjects switched from the high fat to the high carbohydrate diet, and decreased when the change was reversed. These deviations lasted about 10 days and amounted to approximately 2.5 kg; the rate of weight loss after the first 12 days was independent of diet. During the 85% high fat, 1,000 calorie diet, the patients complained about being tired and nauseated at the end of the study, but felt invigorated, yet hungry, after consuming the 91% carbohydrate diet. The variations in weight when changing

diets was found to be due not to alterations in salt content of the diets, but rather to variations in fluid balance.

Werner (1955) found no alteration in weight when changing between a high fat and a high carbohydrate diet, both providing 2875 calories, apart from minor fluctuation in salt and water-balance at the beginning of a dietary period.

Russell (1962), though altering the proportions of fat, carbohydrate, and protein, kept total calories, sodium and water constant. During the high carbohydrate diet, the subjects' weight loss was minimal, while sodium and water-balance were positive; however, during the high fat and protein regimen, they showed an increased weight loss, and the water and sodium balance became negative. Increased water excretion (urinary) does not change the tonicity of body fluids, since it is accompanied by Na^+ excretion.

It seems well established that the short-term increase in weight loss of subjects fed a high fat diet is due to a loss of body water and not to thermogenesis as claimed by Kekwick and Pawan (1957); however, Kasper et al. (1973) support their conclusion. They found that surfeit feeding of a high fat diet (corn and olive oil up to 6,800 calories per day) gave much less weight gain than expected from increased calorie intakes; and also a high weight loss on a diet high in fat but equal in calories compared to one high in carbohydrate.

Recently, Yang and Van Itallie (1976) compared the effects of total fasting, an 800 kcal mixed diet, and an 800 kcal ketogenic diet during 10-day experimental periods. The energy-nitrogen method was

used to quantify the weight loss. The subjects' weight loss was greatest during starvation and intermediate during the ketogenic diet. The nitrogen excretion was a little higher during the ketogenic diet than the mixed diet, but much higher than both during total starvation. Although the percent of fat and water lost was very similar for the ketogenic diet and starvation, the protein loss was much higher during starvation. During the mixed diet, more fat and much less water was lost. The authors concluded that the energy value of body constituents lost during the different periods was almost independent of the dietary regimen, and that the differences in weight loss were almost entirely due to differences in water-balance. This, however, does not necessarily mean that a ketogenic diet causes dehydration. The fat stores appeared to be used primarily on the 800 kcal carbohydrate diet, and they are associated with far less water than are the protein and glycogen stores used during the ketogenic regimen.

Yudkin and Carey (1960) gave an alternative explanation for the greater weight loss seen when a high fat diet is fed. They stated that carbohydrate makes the patient feel hungry, whereas fat gives a feeling of satiety; therefore, the patient eats less on a high fat diet. While this may be the case when patients choose their own food, it cannot explain the shift in water-balance seen in experiments where the caloric intake is controlled.

C. Water Loss During Glucose Deprivation

That water is lost during carbohydrate deprivation seems clear; but the mechanism whereby this occurs is not. During carbohydrate

starvation, glycogenolysis takes place, and this is associated with a significant water loss, since glycogen is stored together with water. When refeeding patients a high carbohydrate diet after depletion of glycogen stores, Olsson and Saltin (1970) found a 2.5 kg weight gain, most of which was probably water, since more than 3 g of water are liberated when 1 g of glycogen is metabolized (Bergström and Hultman, 1972). However, the muscle glycogen stores are only slowly depleted during a carbohydrate-poor dietary regimen of rest (Hultman and Bergström, 1967). So the amount of water released from glycogen depends upon physical activity and the composition of the previous diet; but at most, it can amount to about 2.5 kg (for a 70 kg man). Cahill (1976) states that during the gluconeogenic phase of starvation (first week or so), up to a pound of lean body mass may be lost per day. The loss of lean body mass necessarily gives a corresponding increase in urinary (or sweat) nitrogen loss.

Yang and Van Itallie (1976) found an average protein loss in their subjects of 50 g/day for 10 days of starvation corresponding to a total loss of lean tissue, and glycogen of about 5.0 kg. The fat loss was given as 32.4%, corresponding to 2.4 kg when calculated on an energy deficit basis. Thus, during starvation the glycogen, lean tissue, and fat used can explain the total body weight loss (7.5 kg). Similar calculations made for their ketogenic diet indicate that the mean weight loss was 4.67 kg and the loss from protein, fat and glycogen (assuming 2.5 kg lost through glycogen depletion) was 4.73 kg. Considering the uncertainty of the glycogen loss, there is very good

agreement. If we look at the weight loss during the mixed diet, the actual weight loss was 2.78 kg for the 10 days, and assuming no glycogen loss (which is not necessarily true), the calculated loss is 2.15 kg, which is little more than a pound below the actual loss. This difference can probably be explained by a difference in the size of the glycogen losses.

These calculations show that under the conditions of this study (Yang and Van Itallie, 1976), the weight loss possibly can be explained without assuming any dehydration or shift in the water-balance; however, it is uncertain how much glycogen was actually lost in this experiment.

The short-term weight loss for obese people during starvation is from 10 to 30 pounds, unless they have previously been on carbohydrate restricted diets, whereas for normal man, the initial weight loss is about four to six pounds. While the weight loss in normal persons may be due to glycogen, lean body mass, and fat used for energy, the enormous weight loss reported in obese patients apparently is due to saline diuresis (Cahill, 1976).

D. Possible Mechanisms for Altered Water Balance on Low Carbohydrate Diet

Carbohydrate starvation is known to produce ketosis (Owen and Reichard, 1971a). Since the renal excretion of ketone bodies induces an obligatory loss of sodium (Gamble et al., 1923; and Sartorius et al., 1949), and since sodium is the main regulator of the extracellular volume, it is assumed to cause dehydration (Bloom, 1967).

There are two major objections to this theory. First, the increased excretion of sodium should not lead to a loss of body water

unless the sodium intake is restricted. Actually, the weight loss during a salt free carbohydrate free dietary regimen is no greater than during the same diet containing salt (Bloom, 1967). Spark et al. (1975) have shown that the administration of NaCl or mineralcorticoids has no effect on the weight loss during starvation. It has also been shown that there is a relationship between urinary sodium loss and ketone excretion (Hood et al., 1970).

Secondly, the development of ketosis is gradual, reaching maximal splanchnic production the third day of carbohydrate starvation (Garber et al., 1974), and blood levels continue to rise until the second week (Cahill et al., 1966). Sodium excretion, however, is highest the first day of starvation, and then gradually decreases over the next three to four days (Bloom, 1967; and Gamble, 1923). During the first 24 hours, the sodium loss amounts to 50 to 250 m Eq/day, and then gradually decreases over the next 10 days to as low as 1 m Eq/day (Bloom and Mitchell, 1960). The decrease in sodium excretion is paralleled by a decrease in urinary nitrogen; the magnitude of this reaction depends on the level of protein ingestion prior to the fast (Gilder et al., 1967).

Stinebaugh and Schloeder (1966a) tried to separate the effects of salt restriction and fasting by first feeding a low sodium diet (14 m Eq/day) until the sodium excretion stabilized at less than 20 m Eq/day. The urinary sodium excretion during fasting still increased, showing a peak the fourth day and decreasing to base level about the 10th to the 12th day. So sodium loss during fasting appears to be due partly

to salt withdrawal, and partly to the urinary loss of nitrogen-containing compounds.

In another study, Stinebaugh and Schloeder (1966b) tried to separate the effect of acidosis and fasting by feeding ammonium chloride. They first gave a low sodium diet as before, until urinary excretion was below 20 m Eq/day. Ammonium chloride was then added to produce acidosis similar in degree to that seen during total starvation; when sodium excretion fell to base level, fasting began. In this way they found 338 m Eq of sodium (lost before ammonium chloride was given) due to salt withdrawal, 190 m Eq acidosis (caused by ammonium chloride), and 153 m Eq due to the fast per se. However, if the ammonium chloride produces a mild sodium depletion and fasting produces a sodium surplus from tissue degradation, the kidneys would be expected to retain this surplus sodium during the fast. Veverbrants and Arky (1969), and Haag et al. (1967) found that the administration of bicarbonate did not alter the sodium excretion during starvation; and Gamble (1947) stated that keto acids do not remove fixed base from the body during starvation. The same water loss is generally reported when a diet which is adequate calorically, but low in carbohydrate is fed; such a diet is generally far from restricted in salt. This suggests that if there is any water loss above that from tissue degradation, it appears to be due to carbohydrate deprivation.

It has been demonstrated that the medullary protion of the kidney uses glucose for energy production. A large proportion of the energy expenditure is involved in sodium transport (Ullrich and Marsh,

1963). Wright et al. (1963) have shown that the active sodium transport in the medullary part of the nephron is deficient during fasting. He suggests that there is not enough glucose available for sodium transport during fasting. The kidney is a major gluconeogenic organ, but it assumes this role gradually, after ketosis becomes severe (Owen et al., 1969). The glucose in the blood may not be available to the kidney during starvation when insulin is low, and the water loss during starvation appears to be dependent upon a decrease in the insulin/glucagon ratio (de Fronzo et al., 1975; and Spark et al., 1975). Hoffman (1973) has shown that there is a separate compartment of ATP in the cell wall which is used preferentially for one component of the sodium pump, and it appears that glycolysis is necessary to supply this ATP.

Haag et al. (1967) found that prevention of sodium loss during starvation by feeding carbohydrate is not dependent upon its effect on ketosis, as has also been found by Katz et al. (1968). (Sodium loss during fasting can be interrupted by feeding proteins without preventing ketosis.) The osmotic effect of urea is not the reason for the sodium loss, since a high protein diet increases urea excretion but decreases sodium excretion. Presumably, proteins restore depleted muscle tissue, which retains sodium.

E. Summary

It is well demonstrated that the introduction of a carbohydrate restricting dietary regimen initially produces a greater than expected weight loss. This initial weight loss is primarily due to above normal water loss. Calculations based on results reported in the literature

show that the weight loss observed during starvation and ketogenic regimens can be explained by depletion of glycogen stores, lean tissue degradation, and loss of adipose tissue. Any possible water loss above that from tissue catabolism is in some unknown manner related to carbohydrate deprivation.

IX. LIMITING FACTORS DURING EXERCISE

A. Local Muscle Substrates

1. Triglycerides. The local energy supplying substrates in the working muscle are ATP, phophorylcreatine (PC), glycogen, and triglycerides (Hultman and Bergström, 1973). At 60% of the maximal oxygen uptake and with a work time of 90 minutes (mean), Fröberg et al. (1971) found a decrease in the triglyceride content of the working muscle (from 10.4 to 7.8 mmoles/gm wet muscle). Sixty-one percent of the energy was supplied by carbohydrates and 39% by fat. Of the 39% derived from oxidation of fat, 3/4 came from the triglycerides in the working muscle and 1/4 from the FFA in the blood. It appears that the FFA in the muscle serve a buffering function supplying FFA whenever the demand is higher than the supply from the blood. Fat has never been demonstrated to be a limiting factor during exercise (Hultman and Bergström, 1973).

2. Glycogen. The second largest potential energy store in the muscle is glycogen. It has been shown (Bergström et al., 1967b) that the work capacity is related to the glycogen stores at the beginning of exercise. At low work loads, glycogen was not limiting, since the

glycogen degradation was very slow and the stores are decreased very little (Saltin and Karlsson, 1971b). When the work level was 65 to 89% of the maximal oxygen uptake, glycogen was depleted at the time of exhaustion, and therefore appears to be a limiting factor. Above 89%, the muscle glycogen is not depleted at the time of exhaustion and therefore cannot be the limiting factor. At such high levels of exercise, muscle glycogen decreased rapidly at first, followed by a slower decrease; glucose-6-P and lactate increased markedly at first, then stabilized until exhaustion when a pronounced decrease was seen. Free glucose increased until the end of exercise when a decrease was seen (Bergström et al., 1971a). On this basis, Hultman and Bergström (1973) conclude that the rate of regenerating active phosphate is the limiting factor, and that the pronounced accumulation of lactate and therefore hydrogen ion, is a major factor in bringing about the decreased rate of glycolysis.

Klausen et al. (1973) studied the effect of supermaximal exercise on glycogen utilization. Under such circumstances, part of the energy must come from anaerobic glycolysis. Bouts of supermaximal exercise were alternated with periods of submaximal exercise. In the beginning, blood and muscle lactate increased drastically, but as time went on blood and muscle lactate as well as glycogen decreased, and work time to exhaustion decreased as did the total oxygen uptake in the last bouts of exercise compared to the first. One explanation is that as muscle glycogen decreases, the muscle increasingly uses lactate as substrate, or the red oxidative fibers use the lactate formed by the white glycolytic fibers. Of course the liver extraction of lactate also increases

(Wahren et al., 1971). The decreased substrate availability in combination with the increased intracellular lactate could limit the work done; however, these experiments seem to point to something besides muscle glycogen as a limiting factor. This could possibly be glycogen in the CNS. Essen et al. (1973) have shown that lactate can be taken up by muscles even during severe exercise, and the magnitude of this uptake is related to arterial lactate as well as to intracellular lactate and glycogen concentration.

3. Proteins. Hultman and Bergström (1973) state that protein has never been shown to be used as an energy-producing substrate to any appreciable extent during exercise. It does, however, play some role.

Wahren et al. (1971) studied the splanchnic and leg exchange of substrates during four hours exercise at 30% of the subjects' maximal oxygen uptake. During such exercise, the glucose uptake by the leg at 40 minutes increased to ten times the basal value and to seventeen times basal value at 90 minutes. During the rest of the exercise, a slight decrease was seen, but after four hours exercise, the glucose uptake was still 12 times the basal value.

Alanine was the only amino acid that showed a consistent release during exercise. The muscle output of alanine was slightly increased at 40 minutes, but had risen to three times the basal level after 240 minutes. Net uptake by the leg of the branched chain AA, serine, and citrulline was observed after 240 minutes of exercise. The uptake of oleic acid by the muscle was increased three-fold at 40 minutes of exercise, and it increased an additional 140% by the end of exercise.

The splanchnic glucose output increased two-fold at 40 minutes, and then remained constant until the end of exercise when a small decrease was observed. Splanchnic lactate uptake increased two-fold in the latter part of the exercise, and pyruvate and glycerol uptake increased three- to eleven-fold. Glycerol uptake was the result of a marked increase in the arterial concentration, but the increased lactate uptake was due to an increased fractional extraction. Alanine uptake increased 100% after 240 minutes as a result of augmented fractional extraction; an increased uptake was also seen for threonine, serine, proline and glycine. A significant splanchnic output of valine, isoleucine and leucine was observed after four hours of exercise. The splanchnic fractional uptake of oleic acid increased from 29 to 45%.

These results show that at this level of work, glucose uptake by the muscle increases for the first three hours of exercise, but since fatty acid uptake also increases, the contribution of glucose as a substrate reaches a maximum at 90 minutes under these circumstances. After three hours of exercise, the fat contribution exceeded that of glucose. The combined uptake of glucose and FFA accounted for 65% of the energy utilization at 40 minutes, but at four hours, it had increased to 90%.

Splanchnic glucose output changed little between 40 and 180 minutes, but a slow decrease was seen at the end of exercise. The total glucose output from the liver during the four-hour exercise was estimated to be 75 g; the maximal gluconeogenesis provided 15 to 20 g of the liver glucose output based on splanchnic uptake of lactate, pyruvate,

AA and glycerol. Since the average liver glycogen content in post-absorptive man is around 80 g, about 3/4 of the liver glycogen was mobilized during this exercise. On the basis of substrate balance, the hepatic gluconeogenesis increased from 25% in the postabsorptive state to 40% after four hours of exercise; and increased protein catabolism is partly responsible for this. In many respects, the overall metabolic response to prolonged exercise is strikingly similar to that observed after three days of starvation.

B. Liver Glycogen

Hultman and Nilsson (1971), among others, have shown that hepatic glucose output increases during heavy exercise, and this increase is related to the work load and the duration of exercise. During the terminal part of the exercise, the glucose production increased markedly. At the end of heavy exercise, up to 50% of the glucose consumed by the muscle was released by the liver (Hultman, 1967). At that time, glucose-6-P was low in the muscle, which facilitates utilization of glucose from the liver, because glucose-6-P inhibits hexokinase (an enzyme for phosporylation of glucose).

When strenuous exercise was performed after carbohydrate starvation (depletion of liver glycogen), a pronounced decrease in blood glucose occurred; and in some subjects, severe hypoglycemia was observed, which made them unable to continue the exercise (Bergström et al., 1967). This indicates that under certain conditions (low carbohydrate intake), the work capacity may be limited by the size of the liver glycogen store.

During three days of a carbohydrate-free dietary regimen, the blood sugar did not fall in the resting postabsorptive state; but the concentration of ketone bodies in the blood increased (Fürst et al., 1971). Under such conditions, the CNS apparently switches to ketones as a substrate just as during starvation (Owen et al., 1967; and Owen et al., 1969). When alanine is infused in carbohydrate deprived subjects, there is an immediate increase in glucose production, showing that the low output of glucose is due to insufficient gluconeogenic substrate (Felig et al., 1969c; and Fürst et al., 1971).

When exercise was performed (90% of maximal Vo_2 for 25 minutes) after two days or a carbohydrate rich dietary regimen, a carbohydrate free diet, there were significant differences in liver metabolism. After the carbohydrate free dietary regimen ketogenesis was increased and the uptake of gluconeogenic substrates (alanine, lactate) by the liver was significantly increased, as was the output of glucose. But after a carbohydrate rich dietary regimen, there was no significant uptake of gluconeogenic substrate, and alanine concentration increased in arterial blood. There was no ketone production, but glycogenolysis was about 5 times higher than after the carbohydrate free dietary regimen, where liver glycogen levels were very low (Hultman and Nilsson, 1971).

C. Acid-base Balance

The accumulation of lactic acid and other metabolites during exercise exerts an osmotic effect which may explain the increase in intracellular water observed in the muscle. As lactic acid increases

in the working muscle, there is an increase in the acidity of the cells, which is to a large degree buffered intracellularly. However, it appears that the sodium pump preferentially removes hydrogen ions, and this can explain the increase in sodium seen during exercise (Rooth, 1966; and Bergström et al., 1971a). Lack of available energy in the form of ATP for transport of sodium out of the cell, or increased leakage could also explain these results (Bergström et al., 1971b). Hoffman (1973) has shown that there is a separate compartment of ATP preferentially used by one component of the pump, which appears to be derived from anaerobic glycolysis only. A watershift, per se, is apparently not a limiting factor, since it was most pronounced after five minutes, but work could be continued until depletion of glycogen (Bergström et al., 1971a).

At high work intensities (above 89% of the maximal oxygen consumption), and in isometric work, there is evidence that inhibition of phosphofructokinase limits glycolysis (Bergström et al., 1971b). There is an inverse relationship between lactic acid concentration and the ratio of F-6-diphosphate/fructose-6-P concentration in the muscle; this could mean that the intracellular acidosis limits the availability of glycogen during this kind of exercise. At this level of work, the glycogen stores are not depleted (Bergström et al., 1971a).

Hermansen and Osness (1972) have shown that during maximal continuous, and intermittent exercise, the intracellular pH decreased significantly (from 6.92 to 6.41), while the capillary pH decreased from 7.42 to 6.93. They determined the intracellular pH by taking muscle biopsies and immediately freezing the samples in liquid nitrogen.

Muscle pH down to 6.32 and blood pH values as low as 6.80 were seen (capillary blood). The muscle pH increased rapidly immediately after exercise, whereas the blood pH continued to fall.

With intermittent exercise, blood pH continued to fall in every bout of exercise, but muscle pH increased in the rest periods and fell to about the same value during each work period. These results agree well with those of Karlsson (1971a), who showed that lactate increases to about the same level at exhaustion.

The intracellular pH has a pronounced effect on the metabolic reaction. Hill (1955) in in vitro studies, found that lactic acid formation in response to muscle stimulation ceased at a pH of about 6.3. This supports the theory that substrate availability (anaerobic glycolysis) limits exercise during maximal work. Relman (1972) has shown (in vitro at 37° C) that aerobic lactate production decreases about 60% when pH drops from 7.4 to 6.1, and anaerobic glycolysis falls about 65%. The main reason for this is the sensitivity of phosphofructokinase to ambient pH (the rate limiting enzyme in glycolysis). Relman (1972) also points out that diphosphofructose phosphatase (regulator of gluconeogenesis) is activated by acidosis. Kemp (1969) has shown that a decrease in pH of 0.35 units decreased the maximal velocity of PFK (Phospho Fructo Kinase) from 110 units/mg to 75 units/mg. It appears that hepatic gluconeogenesis is relatively unaffected by the acid-base situation, whereas in the kidney, alkalosis inhibits and acidoses enhances gluconeogenesis (Kamm et al., 1969).

In evaluating the effect of changes in intracellular pH, it is important to take the intracellular compartmentation into account. The data of Carter (1972) indicates that there are marked differences in the pH of the various compartments. The pH values obtained by the muscle biopsy technique must give some kind of weighed average. Carter (1972) suggests that the cytoplasm is the primary compartment involved in discharging acidic metabolic products and in buffering acid or alkali loads added extracellularly. Apart from effecting the availability of substrate, hydrogen ions appear also to have a direct effect of decreasing the muscle contractility (Katz, 1970), and the increase in hydrogen ions reduces the binding capacity of troponin for calcium (Fuchs et al., 1970).

In considering the possible effect that keto acids may have upon the availability of substrate and contractility, it appears unlikely that they can have any significant effect, since ketone bodies are mostly produced in the liver and therefore do not produce the same fall in intracellular pH as the locally produced lactate. However, there is some indication that acidosis and alkalosis produced by administering ammonium chloride and sodium bicarbonate respectively, do affect the work capacity (Denning et al., 1931). It was shown that work capacity decreased after the administration of ammonium chloride. Thus, the ability to neutralize lactic acid would be decreased during ketosis.

Hirche et al. (1973) found that the muscle cell membrane is the main barrier that limits diffusion of lactic acid out of the muscle; and that an increase in bicarbonate concentration in the interstitial

fluid markedly increased the rate of lactate diffusion. It thus appears that a ketogenic diet not only decreases the glycogen stores, but also limits the degree to which glycogen can be used during maximal exercise, since it decreases the bicarbonate level in the blood.

Staib et al. (1964) also found that NaH_2CO_3 improved performance in runners; but Margaria et al. (1971) found that alkalosis induced by several different means, had no significant effect on performance during supermaximal exercise, nor did it affect lactate production.

D. Other Humoral Factors

The possible factors that limit work capacity and duration have received much attention, but it is generally agreed that the limiting factors may vary with the time of exercise, its duration and intensity as well as the training condition of the individual (Haralambie, 1973). However, it is very possible that different factors are limiting in different individuals.

1. Potassium. In short-term exercise, there is a relatively low potassium loss from the muscle (Hultman, 1967); but if the loss is calculated on the basis of dry weight, there is a loss of about 12% after 20 minutes of heavy exercise (Bergström et al., 1971a) due to a shift of water into the cell. Potassium appears to be contained in the glycogen and released as the glycogen stores are used (Hultman, 1967). During long-lasting exercise, plasma potassium decreases (Haralambie, 1973a); this may be due to potassium lost in sweat, or to decreased use of glycogen as work time progresses. However, in 90 km cross-country ski racing, no important changes were seen in plasma potassium concentration (Refsum et al., 1973).

2. Other factors. Numerous other factors have been involved as a cause of fatigue: AMP, IMP, and urea are some of those most frequently mentioned (Haralambie, 1973b). The factors stressed here are those related to substrate utilization.

Since a high-fat diet decreases muscular efficiency (Krogh et al., 1920) and endurance (Christensen and Hansen, 1939), it is possible that these phenomena are related. As some muscle units become exhausted, it is probable that less efficient motor units are recruited with a resultant decrease in efficiency. Krogh et al. (1920) also observed that deficiency decreased most at the time of exhaustion.

E. Summary

Muscle glycogen stores appear to affect the time to exhaustion, when exercise is performed at 65-89% of the maximal oxygen uptake. At lower levels of exercise, muscle glycogen is only used very slowly and does not become depleted at time of exhaustion. At higher levels of exercise, muscle glycogen is not depleted either, and it appears that the intracellular acidosis limits the rate of glycolysis.

The liver glucose output increases during exercise, partly due to increased gluconeogenesis and partly to increased glycogenolysis. The alanine released from the muscle during exercise plays an important role in gluconeogenesis. The utilization of liver glycogen depends upon both the degree and level of exercise. During long-lasting, severe exercise, it is depleted; and the liver glycogen may become a limiting factor under such circumstances.

During high work intensities, there is evidence that the increase in intracellular lactate concentration (which causes a decrease in pH) limits the energy supply to the working muscle, probably through inhibition of phosphofructokinase. The decreased intracellular pH may also directly affect the muscle contractility. Ketosis may decrease the rate of lactate diffusion out of the muscle, and thus indirectly be a limiting factor during certain types of exercise.

EXPERIMENTS

PART ONE: EXERCISE EXPERIMENTS

I. EXPERIMENT I

A. Introduction

The plan for the present study was to determine whether the decreased efficiency, observed by Krogh et al. (1920), and by Marsh and Murlin (1928) when a high fat diet was fed, was due to acidosis from the ketone production. The plan was to feed the subjects a diet high enough in fat to produce ketosis and then to measure the muscular efficiency during work on a bicycle ergometer. The high fat diet would then be repeated with the addition of enough bicarbonate to eliminate the effect of acidosis. By comparing the efficiency under these two conditions, it should be possible to see if the acidosis was responsible for the decreased efficiency observed during a high fat dietary regimen.

It is well established that a high fat diet produces ketosis (Weis and Löffler, 1970; Cahill, 1976; and Krebs, 1966); it is also well documented that a ketogenic diet produces an unexpectedly large weight loss due to a shift in the water-balance (Kekwick and Pawan, 1957; Olesen and Quaade, 1960; Pilkington et al., 1960; and Yang and Van Itallie, 1976). Since it is clear that dehydration impairs performance (Saltin, 1964b; Costill et al., 1974a; and Henschel et al., 1954), it

appeared likely that the ketogenic diet could cause dehydration, and the fatigue associated with dehydration could well be responsible for the decreased efficiency.

B. Procedures

1. Muscular efficiency. Muscular efficiency can be defined as work output divided by energy consumed during the work period, minus the energy expenditure during a similar period of rest. The energy output during work can be measured on a bicycle ergometer or treadmill, and the corresponding energy consumption can be derived from the RQ and the oxygen consumption during work, minus the oxygen consumption during rest.

The oxygen consumption during rest is small relative to the work periods and can be disregarded when the efficiency for two dietary periods are compared. In this study, constant work and recovery periods are employed, and when comparing two such periods, the oxygen consumption is a measure of the efficiency, provided that correction is made for the higher caloric equivalent of 1 liter of oxygen when carbohydrate, rather than fat, is the substrate. In the following, it is therefore to be understood that a change in the oxygen consumption implies a change in the muscular work efficiency.

2. Experimental outline. A general outline of the present experiments is given in Table 1. In order to minimize the effect of training as observed by Krogh et al. (1920), Experiment I began with a three-week period during which the subjects trained for 30 minutes daily on the bicycle ergometer, with work load and speed the same as that used

TABLE 1. Experimental outline showing the sequence of the different diets, the kind and amount of fat and proteins, and the kind of exercise experiments performed.

Experiment	Date[1]	Diet[2]	Fat[3]		Protein[4]		Exercise[5]
			Kind	Amt.	Kind	Amt.	
	3/31-4/18	Ad lib.					Training
	4/21-4/25	CHO I	PUS	15	Soya	10	Ergometer
	4/25-4/27	Ad lib.					None
	4/28-5/ 2	CHO II	PUS	15	Soya	10	Ergometer
I	5/ 2-5/ 4	Ad lib.					None
	5/ 5-5/ 9	Fat I	PUS	75	Soya	10	Ergometer
(1975)	5/ 9-5/11	Ad lib.					None
	5/12-5/16	CHO III	PUS	15	Soya	10	Ergometer
	5/16-5/18	Ad lib.					None
	5/19-5/23	Fat II	PUS	85	Soya	6	Ergometer
	5/23-5/25	Ad lib.					None
	5/26-5/30	CHO IV	PUS	15	Soya	10	Ergometer
	1/18-1/24	CHO I	PUS	10	Soya	5	None
II	1/25-1/30	Fat I	Sat	89	Milk	5	None
	1/30-2/ 7	CHO II	PUS	10	Soya	5	None
(1976)	2/ 8-2/13	Fat II	PUS	89	Soya	5	None
	2/13-2/20	CHO III	PUS	10	Soya	5	None
	4/ 4-4/17	CHO I	PUS	25	Soya	15	Treadmill
III	4/18-4/29	Fat I	Sat	80	Meat	15	Treadmill
	4/29-5/16	CHO II	PUS	25	Soya	15	Treadmill
(1976)	5/17-5/28	Fat II	PUS	80	Soya	15	Treadmill

[1]The dates overlap because blood samples were taken in the postabsorptive state at the end of each experimental period, the same morning the new dietary regimen was introduced.

[2]"Ad lib." indicates that the subjects were free to choose their own diet (Friday and Saturday); however, during the second and third experiments, they changed directly from one diet to the next. "CHO" indicates the high carbohydrate control diet to which the results for the high fat periods were compared.

continued

TABLE 1--continued

[3]"PUS" indicates that the fat came primarily from corn oil and avocadoes rich in PUS; "Sat" indicates that the fat came from either milk or meat. The amount of fat is given as percent of calories.

[4]The primary source of protein is indicated; however, other constituents of the diets also contributed some protein. The amount of protein is given as percent of calories.

[5]"Training"--the subjects trained for 30 minutes daily on the bicycle ergometer, but no expired air was collected.

"Ergometer"--the subjects worked 10 minutes on the bicycle ergometer on the first, third, and fifth days of the experiment. (During the first "ergometer" period, the procedure was the same, but no results were collected.)

"None"--no exercise was performed, and the normal activity of the subjects was kept as uniform as possible.

"Treadmill"--a standard walk (5 minutes) and a maximal oxygen consumption experiment were carried out with one of the subjects (KB) on each Wednesday of the experimental periods.

in the following study periods. Each dietary period lasted five days, and on Friday and Saturday between periods, the subjects were allowed to choose their own food (except for alcoholic beverages). No data was collected during the first high carbohydrate control diet (CHO I), although the procedures were exactly the same as during the other control periods (CHO II-IV).

A second high carbohydrate control period was followed by the Fat I diet using about 75% of the calories from fat. After another control period (CHO III), the Fat II diet was introduced, using about 85% of calories from fat. Experiment I then terminated with a fourth control period (CHO IV).

3. Subjects. The subjects participating in these studies were William D. Hart (WH), Rong-Ching Hsieh (RH), and Kristian Balwin (KB). Weight (Kg), height (cm), and birth-dates are given in Table 2, the approximate energy needs are also shown.

TABLE 2. Weight, height, and birth-dates for the subjects.

Subject	Weight (Kg)	Height (cm)	Birth-date	Energy Needs (Kcal)[1]
WH	104	188	1945	2700
RH	61	175	1950	2300
KB	64	175	1935	2400

[1]Approximate energy consumption.

KB and RH are very similar in build and weight, both on the low side of the "desirable weight" standards (prepared by Metropolitan Life Insurance Company, 1960). KB is from Denmark and RH from Taiwan. WH is American, but of Scandinavian origin, heavier build, and about 50 lb. above the "desirable weight". WH had a history of developing high uric acid levels when consuming high fat diets, and for this reason did not participate in Experiment III. Therefore, RH became the second subject. For family reasons WH did not participate in CHO III and the first day of CHO IV in Experiment I.

4. Diets. The composition of the diets was calculated as shown in Appendices A, B, C, and D, and as given in Table 3. The aim of the high fat diet was to produce ketosis. The level of fat to accomplish this was not precisely known, so the first high fat diet contained 75% of the calories from fat, which was increased to 85% in the second high fat diet period. Proteins contributed 10% of the calories in the control diet and the first high fat diet, but in the second high fat diet, the protein level was decreased to 6% of calories in order to make the diet more palatable. The composition of the diets was calculated based on information from the manufacturer where possible, and otherwise based on information in the Agriculture Handbook, No. 8 (Watt and Merrill, 1975). The subjects ate three different meals a day and received a little snack to take home. In order to decrease variation in composition throughout the study, food was bought in one lot and refrigerated or frozen until used. The subjects ate the same food in the same amount every day. The food was prepared and eaten in the dietary kitchen of

TABLE 3. Approximate composition of diets (percentage of calories) in Experiment I.

Diet	Carbohydrate	Fat	Protein
Control	75	15	10
Fat I	15	75	10
Fat II	9	85	6

the Food Science Building. The composition of the various diets is given in the above table. KB received a total of 2400 kcal and WH received 2700 kcal daily. The subjects also received one multivitamin and mineral tablet/day.[1] The fat in the high fat diets was basically

[1] Unicap, made by the Upjohn Company, Kalamazoo, Michigan 49001. The composition of these tablets were:

Vitamins	
Vitamin A	1.5 mg
Vitamin D	10 µg
Thiamine mononitrate	10 mg
Riboflavine	10 mg
Ascorbic acid (Na-ascorbate)	300 mg
Niacineamide	100 mg
Pyridoxine Hydrochloride	2 mg
Calcium Panthothenate	20 mg
Cobalamine concentrate	4 µg
Vitamin E	30 int. units

Minerals	
Fe (as ferrous sulfate)	10 mg
I_2 (as potassium iodate)	.15 mg
Ca (carbonate)	50 mg
Cu (sulfate)	1 mg
Mu (sulfate)	1 mg
Mg (sulfate)	6 mg
K (sulfate)	5 mg

from corn oil and avocadoes, whereas the majority of the protein was soy-protein made by Worthington Company.[1] In the control diet, soy-protein also supplied the larger share, but some protein came from potatoes and bread used in this diet. Most of the carbohydrate was in the form of fruits and vegetables. No coffee, tea, or alcohol was allowed.

5. Exercise. Exercise was performed on a bicycle ergometer at 70 rpm/min and constant load. Subjects arrived at the laboratory at 7:00 a.m. in the postabsorptive state and exercised for 10 minutes at about 50% of the maximal oxygen consumption (based on the maximal oxygen consumption as determined for KB in Experiment III); this exercise was followed by a 10-minute recovery period. Exercise was performed on Monday, Wednesday, and Friday of each experimental period. On Tuesday and Thursday, measurements were taken in the resting state only, at least 12 hours after the last meal, and after 20 minutes rest in the laboratory. The measurements were taken with the subjects sitting in a chair at ambient room temperatures.

6. Measurements. The water intake was recorded in a log book for water-balance calculations. Body weight measurements were taken nude in the morning after urination. An attempt was also made to measure the weight loss during exercise to get an idea of the sweat loss. Twenty-four-hour urine samples were collected for pH and ketone body measurements, and the urine was kept refrigerated under toluene

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[1]Worthington Foods, Worthington, Ohio.

during the day of collection. When the 24-hour collection was complete, pH and volume were measured immediately, and samples frozen. Friday, the last day of each experimental period, duplicate venous blood samples were taken before and immediately after the exercise period. The blood samples were taken anaerobically, placed on ice, and delivered to the Olin Health Center where they were analyzed for pH and pCO_2.

The expired air was collected during the entire exercise periods. Each collection bag represented two minutes and the change of bags was done automatically when the operator pushed a button. The expired air was also collected for the first 10 minutes of the recovery period; two bags were used--one for the first three minutes, and one for the last seven minutes. Heart rate was measured for both work and recovery periods.

7. Equipment. The pH and pCO_2 were measured on a Radiometer pH-meter using glass electrodes. Weight was measured on a scale (Fairbanks FN-42) accurate to $\pm$ 20 g. Exercise was performed on a modified Schwin electromagnetic bicycle ergometer equipped with a 12-volt automobile alternator. The load was regulated by changing the resistance of the output and the same setting was used for both subjects. The load is independent of the pedaling speed. The expired air was collected through a low resistance Otis-McKerrow respiratory valve and the modified Douglas method was used in collecting the gas. The heart rate was counted on a Well's pulse rate meter with readout to a Gibson five channel recorder.

The oxygen content of the expired gas was analyzed on a Beckman paramagnetic analyzer (model E2); and the carbondioxide was analyzed on a Beckman infrared analyzer (model LB 15A). For calibration of the gas analyzers, the zero point was set with helium, and for the upscale values, atmospheric air and a standard gas were used. The composition of the standard gas corresponded to the expired gas (about 4.3% CO_2 and 17.73% O_2). The standard gas sample was calibrated with repeated Haldane gas analysis.

8. Calculations and analysis. The expired air was collected and immediately analyzed for CO_2 and O_2, the volume was measured and the temperature recorded. The barometric pressure in the laboratory was also recorded at the time of analysis. The expired gas volume was then converted to STPD (0 C, 760 mm Hg. dry.) conditions using the method of Consolazio et al. (1963). By using the measured concentrations of CO_2 and O_2, the RQ and the true oxygen consumption were determined from the monograms and formulas described by Consolazio et al. (1963).

C. Results

1. Oxygen consumption and RQ during rest. Calculated RQ and oxygen consumption during rest are shown in Table 4.

2. RQ and oxygen consumption during exercise. The RQ always increased to a fairly steady level during the first three minutes or so of exercise, although a decrease was often observed at the end of the work period. The RQ, as expected, was lower during the high fat period than during the high carbohydrate period. The RQ's from two typical work periods are plotted in Figure 1. A typical plot is shown for each period (fat and carbohydrate) for each subject.

TABLE 4. RQ and oxygen consumption during rest in Experiment I.

Date	Diet	RQ[3]	O_2 Conc. ml/min[3]	RQ[4]	O_2 Cons. ml/min[4]
4/29[1]	CHO II	0.76	270	0.78	310
5/ 1[2]	CHO II	0.83	330	0.74	300
5/ 6[1]	Fat I	0.77	260	0.81	290
5/ 8[2]	Fat I	0.77	276	0.72	330
5/13[1]	CHO III	0.84	271		
5/15[2]	CHO III	0.85	256		
5/20[1]	Fat II	0.75	340	0.78	320
5/22[2]	Fat II	0.70	273	0.72	298
Mean	CHO[5]	0.82	282	0.76	305
Mean	Fat[6]	0.75	287	0.76	310

[1]Second day of the dietary period.

[2]Fourth day of the dietary period.

[3]Results for KB.

[4]Results for WH.

[5]Mean of all CHO periods.

[6]Mean of all Fat periods.

FIGURE 1. RQ's during exercise for KB and WH during Experiment I. (The RQ's are plotted as a function of time for each subject for a typical work period during a high fat and a high carbohydrate regimen. The value plotted for zero time is the resting RQ measured on a different day.)

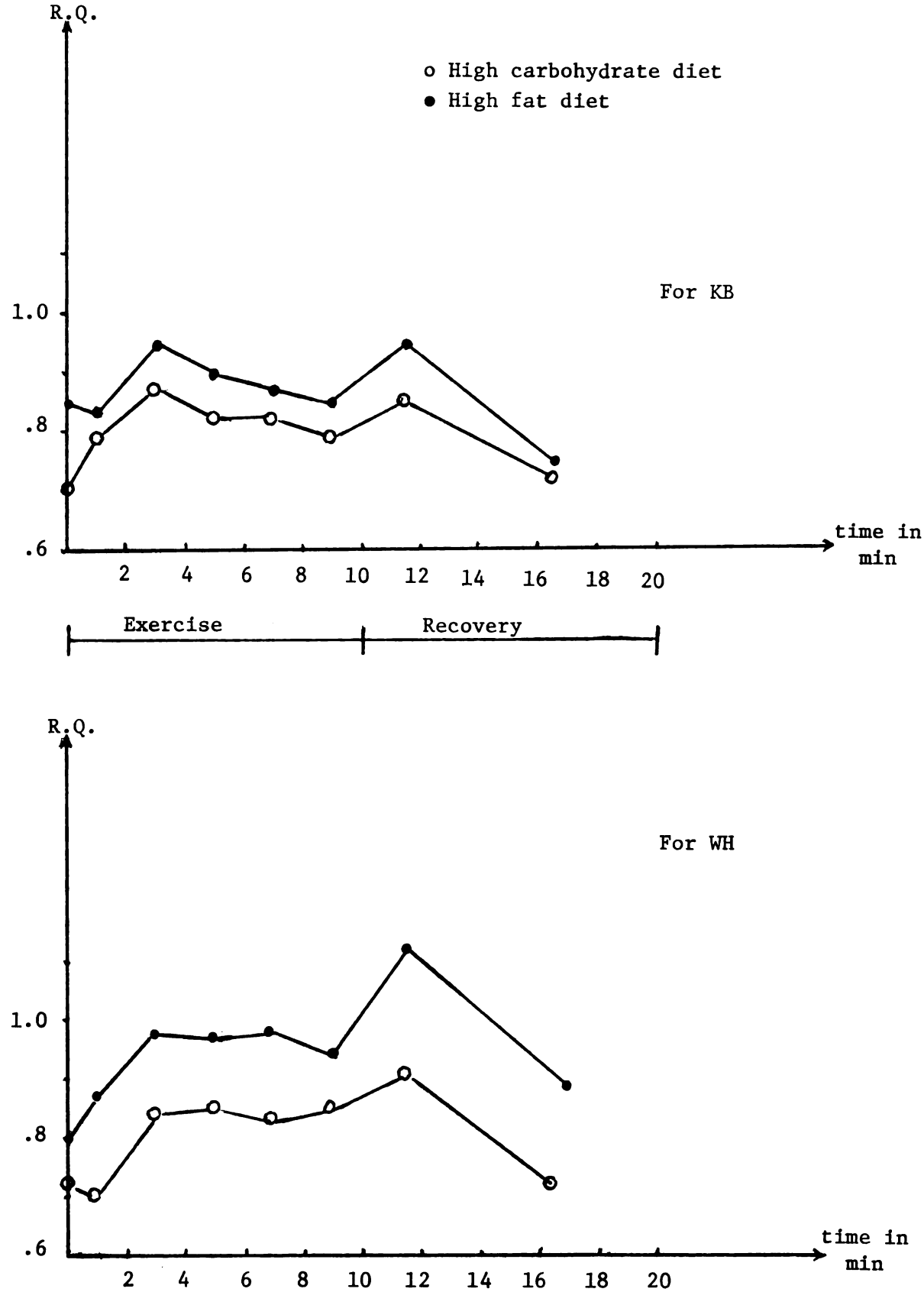
R.Q.
o High carbohydrate diet
● High fat diet
For KB
1.0
.8
.6
time in min
2 4 6 8 10 12 14 16 18 20
Exercise
Recovery
R.Q.
For WH
1.0
.8
.6
time in min
2 4 6 8 10 12 14 16 18 20

FIGURE 1

Since apart from the first four minutes of work, the RQ was fairly stable, the mean of the next three 2 min periods has been calculated. The total oxygen consumption during work and recovery in each dietary period has been calculated, and is given as work and recovery O_2. Since the oxygen consumption during the first four minutes usually was less than the rest of the work period, the work oxygen is divided into Initial O_2 (first four minutes) and Main O_2 (last six minutes). In order to minimize daily variations, the mean oxygen consumption is calculated for each dietary period. The results are shown in Table 5 for KB and in Table 6 for WH. The mean RQ for each dietary period corresponding to the main oxygen consumption is also calculated and shown in Tables 5 and 6. The maximal pulse rates are likewise given.

3. Blood data. On the last day of most dietary periods, two venous blood samples were taken before and after exercise for measurement of pCO_2 and pH. The mean values of two determinations for KB are given in Table 7.

4. Body weight. The body weight was measured throughout the study (nude), but on the days of exercise, it was recorded before and after exercise. The results are shown in Table 8 for KB and in Table 9 for WH.

The average weight losses during exercise for the fat and carbohydrate periods were calculated as was the total weight loss during the fat periods. The results are shown in Table 10.

TABLE 5. Pulse rate, RQ, and oxygen consumption during exercise, for KB, in Experiment I.

Date	Diet	PR[1]	RQ[2]	Total[3]	Work[4]	Rec.[5]	Int.[6]	Main[7]
4/28	CHO II	148	.86	30.90	25.51	5.39	9.84	15.67
4/30	CHO II	148	.86	29.76	24.12	5.64	9.26	14.86
5/ 2	CHO II	152	.87	30.38	24.15	6.23	9.18	14.97
Mean	CHO II	149	.86	30.35	24.59	5.75	9.43	15.17
5/ 5	Fat I	148	.83	31.54	25.20	6.34	9.58	15.62
5/ 7	Fat I	147	.83	29.30	23.51	5.79	8.89	14.62
5/ 9	Fat I	156	.82	32.60	25.18	7.42	9.47	15.71
Mean	Fat I	150	.83	31.15	24.63	6.52	9.31	15.32
5/12	CHO III	144	.91	29.55	24.00	5.55	8.88	15.12
5/14	CHO III	144	.98	27.61	22.34	5.27	8.33	14.01
5/16	CHO III	147	.88	30.20	24.40	5.80	8.83	15.57
Mean	CHO III	145	.92	29.12	23.58	5.54	8.68	14.90
5/19	Fat II	152	.87	30.11	24.46	5.65	9.39	15.07
5/21	Fat II	144	.89	28.78	22.72	6.06	8.50	14.22
5/23	Fat II	159	.81	29.34	22.59	6.74	9.13	13.46
Mean	Fat II	152	.86	29.41	23.26	6.15	9.01	14.25
5/26	CHO IV	147	.89	28.33	22.50	5.83	8.46	14.04
5/28	CHO IV	145	.87	29.26	23.79	5.47	8.72	15.07
5/30	CHO IV	150	.89	29.45	23.72	5.73	9.22	14.50
Mean	CHO IV	147	.88	29.01	23.34	5.68	8.80	14.54

[1]Pulse rate (beats/min), at the end of exercise.

[2]The mean RQ of the last six minutes of exercise.

[3]Total oxygen consumption (liter/20 min).

[4]Oxygen consumption during work (liter/10 min).

[5]Oxygen consumption during recovery (liter/10 min).

[6]Initial oxygen consumption during the first four minutes (liter/4 min).

[7]Main oxygen consumption during the last six minutes (liter/6 min).

TABLE 6. Pulse rate, RQ, and oxygen consumption during exercise, for WH, in Experiment I.

Date	Diet	PR[1]	RQ[2]	Total[3]	Work[4]	Rec.[5]	Int.[6]	Main[7]
4/28	CHO II	148	.95	31.10	24.81	6.29	8.76	16.05
4/30	CHO II	140	.85	31.27	24.78	6.49	8.24	16.54
5/ 2	CHO II	144	.92	33.42	25.07	8.35	9.27	15.80
Mean	CHO II	144	.91	31.93	24.89	7.04	8.76	16.13
5/ 5	Fat I	148	.84	32.67	25.92	6.75	9.24	16.68
5/ 7	Fat I	150	.86	29.67	23.54	6.13	9.15	14.39
5/ 9	Fat I	159	.86	36.28	29.03	7.25	11.16	16.87
Mean	Fat I	152	.85	32.87	26.16	6.71	9.86	16.31
5/19[8]	Fat II	147	.90	31.48	25.21	6.27	8.96	16.25
5/21	Fat II	162	.89	30.25	24.92	5.33	9.14	15.78
5/23	Fat II	171	.84	33.11	26.21	6.90	9.55	16.66
Mean	Fat II	160	.88	31.61	25.45	6.17	9.22	16.23
5/28[9]	CHO IV	144	.96	29.77	23.88	5.89	8.72	15.16
5/30	CHO IV	156	.94	29.50	23.47	6.03	9.01	14.46
Mean	CHO IV	150	.95	29.64	23.68	5.96	8.87	14.81

[1]Pulse rate (beats/min).

[2]The mean RQ of the last six minutes of exercise.

[3]Total oxygen consumption (liter/20 min).

[4]Oxygen consumption during work (liter/10 min).

[5]Oxygen consumption during recovery (liter/10 min).

[6]Initial oxygen consumption during first 4 min (L/4 min).

[7]Main oxygen consumption during the last 6 min (L/6 min).

[8]WH did not participate in the previous control period.

[9]WH did not participate the first day of the period.

TABLE 7. Blood pH and pCO_2 for KB in Experiment I.

Date	Diet	pH^1	pH^2	ΔpH^3	$pCO_2{}^4$	$pCO_2{}^5$	$\Delta pCO_2{}^6$
5/ 9	Fat	7.34	7.30	0.04	55.3	51.2	4.1
5/16	CHO	7.29	7.28	0.01	59.6	42.9	16.7
5/23	Fat	7.36	7.32	0.04	51.5	47.8	3.7

[1]pH before exercise.

[2]pH after exercise.

[3]Decrease in pH during exercise.

[4]pCO_2 before exercise.

[5]pCO_2 after exercise.

[6]Decrease in pCO_2 during exercise.

TABLE 8. Body weight and weight loss during exercise for KB in Experiment I (all values in kg).

Date	Diet	$W_1{}^1$	$W_2{}^2$	ΔW^3
4/28	CHO II	65.68	65.62	0.04
4/30	CHO II	65.50	65.40	0.10
5/ 2	CHO II	65.54	65.32	0.22
5/ 5	Fat I	66.06	65.88	0.18
5/ 7	Fat I	65.76	65.64	0.12
5/ 9	Fat I	64.82	64.13	0.69
5/12	CHO III	65.54	64.90	0.64
5/14	CHO III	65.44	65.32	0.12
5/16	CHO III	65.56	65.35	0.11
5/19	Fat II	65.14	64.40	0.74
5/21	Fat II	64.96	64.76	0.20
5/23	Fat II	64.00	63.74	0.26

[1]Body weight before exercise.

[2]Body weight after exercise.

[3]Body weight loss during exercise.

TABLE 9. Body weight and weight loss during exercise for WH in Experiment I (all values in kg).

Date	Diet	W_1[1]	W_2[1]	ΔW[1]
4/28	CHO II	99.64	99.34	0.30
4/30	CHO II	99.66	99.22	0.44
5/ 2	CHO II	99.18	98.88	0.30
5/ 5	Fat I	99.78	99.62	0.16
5/ 7	Fat I	98.66	98.18	0.48
5/ 9	Fat I	97.66	97.42	0.24
5/19	Fat II[2]	99.66	99.30	0.36
5/21	Fat II	97.68	97.36	0.32
5/23	Fat II	97.14	96.92	0.22

[1]For explanation of abbreviations see Table 8.

[2]WH did not participate in the second control period because of a funeral.

TABLE 10. Mean weight loss in kg (from Tables 8 and 9) during Experiment I.

Diet	Total weight loss		Average exercise loss	
CHO II	0.00[1]	0.46[2]	0.21[1]	0.35[2]
Fat I	1.24[1]	2.12[2]	0.33[1]	0.29[2]
Fat II	1.14[1]	2.52[2]	0.40[1]	0.30[2]

[1]Results for KB.

[2]Results for WH.

5. Water-balance data. Water-balance is used to indicate the difference between water intake and urinary output. Tea, coffee, and soft drinks were not permitted. The daily urine volume was recorded, and the total water intake calculated from the record of drinking water consumed, plus the water contained in the food. The metabolic water is ignored; although on a weight basis, fat produces more water, on a caloric equivalent basis, carbohydrate produces slightly more water. The water-balance represents the water lost by perspiration and other losses. These data and the urinary pH are shown in Table 11 for KB, and the mean values in Table 12.

6. Urinary ketone excretion. The subjects' urinary ketone excretion was evaluated with Keto-Stix (Ames) based on the purple color developed when the ketone bodies react with nitroprusside. The ketone level is graded as "small", "medium", and "high". During the Fat I regimen (75% of calories from fat), the tests were negative, except for an occasional, very weak reaction toward the end of the 5-day period. During the Fat II regimen (85% of calories from fat), a trace of ketones was found in the urine on the second day, which increased to medium on the last day. The results for Fat II regimen are tabulated in Table 13. There was no significant difference between the subjects.

TABLE 11. pH and water-balance data for KB during Experiment I.

Date	Diet	Water intake[1]	Urine	Water-balance[2]	pH[3]
4/28	CHO II	2338	1000	1338	7.00
4/29	CHO II	2588	900	1688	7.05
4/30	CHO II	2838	690	2148	6.67
5/1	CHO II	2088	900	1188	6.95
5/2	CHO II	2388	850	1488	7.09
5/5	Fat I	2296	1319	906	6.95
5/6	Fat I	2046	1250	796	6.86
5/7	Fat I	1796	950	850	6.58
5/8	Fat I	2046	890	546	6.66
5/12	CHO III	2338	920	1418	6.96
5/13	CHO III	2588	810	1778	7.20
5/14	CHO III	2838	890	1948	6.83
5/15	CHO III	2838	530	2308	6.50
5/19	Fat II	1428	490	938	6.07
5/20	Fat II	2428	540	1888	6.10
5/21	Fat II	2428	580	1848	6.61
5/22	Fat II	2928	1170	1758	6.25

[1]Water intake consists of the water contained in the food and the water or other fluid intake.

[2]Water-balance is calculated as the water intake minus the urine volume = water lost in stool, through perspiration, and as insensible water loss.

[3]pH of 24-hour urine collection.

TABLE 12. Mean water-balance and urinary pH for KB during Experiment I.

Diet	Mean water-balance[1]	Mean urine pH
CHO II	1570	6.95
Fat I	775	6.76
CHO III	1868	6.87
Fat II	1608	6.26

[1]Mean water-balance in ml, calculated as the difference between water intake and urine.

TABLE 13. Urinary ketone levels above control values during the Fat II regimen in Experiment I.

Date	Ketone level[1]
5/19	0
5/20	trace
5/21	small
5/22	small-to-medium
5/23	medium

[1]Graded from 0 (control value) to high (severe ketosis).

II. EXPERIMENT III (EXERCISE)

A. Introduction

As can be seen from the RQ's in the previous experiment (Tables 5 and 6), there was not much difference between the substrate utilized during exercise when the fat or carbohydrate diet was consumed. This was no doubt due to the utilization of muscle glycogen. In subject KB, however, a significant decrease was seen in the RQ in the last experiment during the Fat II regimen in Experiment I; this probably indicates a functionally important decrease in muscle glycogen. Also, the exercise RQ's during the Fat II period generally were higher than those for the Fat I regimen, despite the fact that Fat II contained more fat (85% vs. 75%). This may well have been due to increased glycogen stores at the initiation of the Fat II diet. Partial depletion of the glycogen during the Fat I regimen might have caused supercompensation of the glycogen stores when the high carbohydrate diet (CHO III) was fed.

There are two possible ways to accomplish a higher utilization of fat. First of all, one can increase the level and particularly the duration of the exercise to deplete the glycogen stores, or the glycogen stores can be depleted prior to the experiment. The latter approach is undesirable because it causes undue hardship on the subjects, as will be discussed later. More importantly, perhaps, depletion of muscle glycogen stores may well be the factor that causes the decreased efficiency when a high fat diet is fed. At low work levels fat is the major substrate (Havel, 1971), and muscle glycogen is not the limiting factor (Saltin and Karlsson, 1971). It therefore seems possible to increase

the utilization of fat by decreasing the work load.

Another exercise experiment was carried out with the subject KB at a lower exercise level. In this series, a treadmill was used instead of the bicycle ergometer, but otherwise, the methodology was the same except for shorter work and recovery periods. The composition of the diets is given in Table 14 as percent of calories, and the calculations are shown in Appendices E, F, G, and H.

TABLE 14. Approximate composition of the diets as percent of calories during Experiment III.

Diet	Protein	Carbohydrate	Fat
High CHO	15	60	25
High Fat	15	5	80

In contrast to the bicycle experiment, which was done in the postabsorptive state, the treadmill experiment was done in the absorptive state (1-3 hours after the meal); this appears to favor utilization of the substrate fed (fat on a high fat diet). The speed and grade of the treadmill could be varied, and in addition to determining the maximal oxygen consumption, a standard walk experiment was performed.

B. Procedures

The experimental outline can be seen from Table I (page 97). The standard work was done by walking on the treadmill at 3 1/2 mph for five minutes, followed by a recovery period of five minutes. Because

of the relatively short periods, important variations in oxygen consumption can be expected when the two diets were fed, due to differences in lactate production and rate of recovery. Treadmill work in general, is less precise than work on the bicycle ergometer. No training period preceded these experiments, but the subject KB was in good physical condition. The maximal oxygen consumption was determined immediately after the recovery period for the standard run, which served as a warm-up period. Determination of the latter involved a standard run on the treadmill on the level for five minutes at 6 mph; the speed was then increased to 7.5 mph and maintained for two minutes. The expired gas collection started at the end of the standard run and continued until exhaustion (bag changed every 30 seconds). The grade was increased 2.5% each minute until the subject became exhausted. Air collection and analysis were done in the same way as during Experiment I.

C. Results

1. Standard work. The mean RQ and oxygen consumption (during work, recovery, and total), as well as the maximal heart rate for the different dietary periods are shown in Table 15. The average oxygen consumption for the work period during the high fat diet was 7.58 liters (per 5 minutes), compared to 6.46 liters (per 5 minutes) for the high carbohydrate diet. For the total oxygen consumption, the corresponding values were 10.08 and 8.96, respectively.

2. Maximal oxygen consumption. The maximal oxygen consumption, average RQ, and maximal heart rate (beats/min) are given in Table 16.

TABLE 15. Oxygen consumption, RQ and heart rate for KB during standard walk in Experiment III.

Date	Diet	Work O_2[1]	Recovery[1]	Total O_2	RQ	HR[2]
4/14	CHO I	6.95	2.71	9.66	0.86	120
4/27[3]	Fat I	7.63	2.41	10.03	0.70	121
5/ 5	CHO II	6.28	2.80	9.08	0.87	115
5/12	CHO II	6.16	1.98	8.13	0.95	105
5/19	Fat II	7.05	3.06	10.11	0.62	110
5/26	Fat II	8.06	2.05	10.11	0.64	110
Mean[4]	Fat	7.58	2.51	10.08	0.65	114
Mean[5]	CHO	6.46	2.50	8.96	0.89	113

[1]Liter/5 min.

[2]Mean heart rate for one minute at the end of exercise.

[3]The measurements on 4/28 were discarded because of error in measuring the volume of expired air.

[4]Mean of all the fat periods.

[5]Mean of all the carbohydrate periods.

Table 16. Maximal oxygen consumption and heart rate for KB during Experiment III.

Date	Diet	Max. V_{O_2}[1]	RQ	Max. HR[2]
4/14	CHO I	4.28	0.92	194
4/28[3]	Fat I	4.48	0.90	190
5/12[4]	CHO II	3.96	1.19	185
5/19	Fat II	4.48	0.84	184
5/26	Fat II	4.32	0.78	187

[1]Liter/min.

[2]Heart rate at end of exercise (beats/min).

[3]No determination on 4/21.

[4]Data not available for 5/5 due to faulty gas collection.

III. EVALUATION OF DATA

A. Experiment I

1. Resting oxygen consumption and RQ. There is a fairly great variation in the resting oxygen consumption as seen from Table 4 (page 105); the two main reasons for this are probably variation in the temperature of the room where the measurements were taken, and the degree to which the subjects were relaxed on the different days. The means are shown in Table 4, as are the corresponding mean RQ's. The inter individual variation seen in this table is partly explained by the difference in body mass (on a weight basis, the value is less for WH).

The resting oxygen consumption does not appear to depend upon the dietary regimen. Although the average RQ for KB is lower when the

high fat diets were eaten, there is no difference for WH. This is not surprising, since in the resting postabsorptive state, the primary substrate is fat, regardless of diet. The last determination of the resting RQ was made on the next to the last day of each dietary period; this RQ was 0.70 for KB and 0.72 for WH, which is close to pure fat metabolism. Only KB responded with an increased carbohydrate metabolism in the postabsorptive state during the high carbohydrate diet. The low resting RQ's agree well with the results obtained using radioactive tracers (Havel, 1971). From such studies, it appears that glucose contributes no more than 20% of the substrate; this corresponds to a RQ of 0.76 (Swift and Fisher, 1964). It is interesting to notice that in KB, the high carbohydrate diet caused an increase in the utilization of CHO in the resting state (about 50% at a RQ of 0.85). In recent studies, Felig et al. (1975) showed by catheterization techniques, that the liver is the primary site of disposal of an oral glucose load; and some of the glucose retained by the liver is undoubtedly released later for peripheral utilization.

2. Exercise RQ. The exercise RQ's are not consistently lower during the high fat dietary regimen compared to the high carbohydrate diet; but on the last day of exercise during the high fat period, the RQ begins to decrease (Tables 5 and 6, pages 109 and 110). During exercise of this length (10 min), glucose plays an important role as substrate (Havel, 1971); but during the high fat period, the liver glycogen will be depleted (Nilsson and Hultman, 1973), whereas one would not expect the muscle glycogen to be exhausted (Hultman and

Nilsson, 1975). However, the relatively lower RQ on the last day of exercise indicates that even with a relatively low activity level, the muscle glycogen is significantly decreased. The work RQ's obtained during the high fat period are considerably higher than those of Christensen and Hansen (1939); but their data were based on exercise of long duration, and they saw a continual decrease in the RQ's with time. For KB, the RQ's are only slightly higher than those observed by Marsh and Murlin (1928), who used a work time of 20 minutes. Based on the RQ, fat contributed (for KB) from 60-63% of the calories on the last day of the high fat dietary periods (Swift and Fisher, 1964). This shows the importance of the muscle glycogen during exercise. During the high carbohydrate periods, fat contributed on the average, about 35% of the calories during exercise; but in some experiments, as little as 6% of the calories came from fat.

In both KB and WH the RQ appears to be lower during the Fat I period than during the Fat II period (Tables 5 and 6). This may be due to carbohydrate supercompensation during the CHO III period between the two high fat periods (Hultman and Nilsson, 1975).

3. Efficiency and oxygen consumption. Krogh et al. (1920) calculated the muscular work efficiency by subtracting the resting oxygen consumption from the oxygen consumption during work (converted to the caloric equivalent). However, since the variation in the oxygen consumption during work is greater than the resting oxygen consumption, the latter is just as good a measure of efficiency. The oxygen consumption varies only slightly with the substrate used, so on a normal diet, no

corrections need be made. However, the caloric value of 1 liter of oxygen is 7.7% higher when pure carbohydrate rather than fat is the substrate (Swift and Fisher, 1964). If we assume that the caloric requirement for a given task is independent of the substrate used, this means that the oxygen consumption will decrease 7.15% when subjects change from pure fat to pure carbohydrate as the energy substrate. Looking at Table 5, it is seen that on 5/14 (CHO III), fat contributed about 6% of calories, and on 5/23 (Fat II), the contribution was about 61%, an increase of 55%. The oxygen consumption increased about 6% compared with an expected increase of 4.3% based on the caloric equivalents; this difference is within experimental error.

If we look at the total oxygen consumption for KB, we can clearly see the effect of training at the beginning of Experiment I (4-5%) between the first two carbohydrate diets (CHO II and CHO III); but thereafter, very little, if any effect is seen. This agrees with the observations of Krogh et al. (1920).

Looking at the total oxygen consumption for KB, there is very little increase in the oxygen consumption during the high fat dietary regimen; the increase is 1-3%, which is to be expected because of the lower caloric value of oxygen when fat supplies the energy. This difference practically disappears when we look at the oxygen consumption for either the total work period or the last six minutes. During recovery period, the oxygen consumption is about 10% higher during the high fat periods, and it is about 4% higher during the first four minutes of exercise. These data suggest that the substrate has no effect on the

oxygen consumption when caloric equivalents are considered; however, there appears to be a trend toward less anaerobic glycolysis during the high fat periods.

The venous pH (Table 7, page 111) surprisingly, was higher (0.04) during the high fat periods, but after exercise, the difference was insignificant (0.02) because of less decrease during the exercise. The pre-exercise pCO_2 is somewhat higher during the high carbohydrate periods, but it becomes much less after exercise. The decrease was about four times greater during the high carbohydrate periods, which also indicates a higher lactate production and/or a decreased buffering capacity. Because of variation in analysis from day to day, the differences in pH and pCO_2 between pre- and post-exercise values during the diets are probably more meaningful than the absolute values.

The urinary ketone body excretion increased progressively (Table 13, page 115); but apparently, the blood is well buffered, since the venous pH did not appear to decrease (see Table 7, page 111). As will be described later, in Experiment II the arterial blood pH was about 0.03 units lower during the high fat periods. Sutton et al. (1976) has found that a higher pH (alkalosis) favors anaerobic glycolysis, which is supported by the increase in anaerobic glycolysis seen during the high carbohydrate period. The slow development of ketosis agrees well with the results of Garber et al. (1974); and Bloom (1967). A decrease in the urinary pH is also seen when fat diets are consumed (Table 11, page 114).

4. Weight loss. There is no difference in the mean body weight loss during exercise (Table 10, page 112) during the fat and carbohydrate periods for WH; but for KB, the fat periods appear to be associated with a higher weight loss during exercise. Both Krogh et al. (1920), and Marsh and Murlin (1928) report perfuse sweating during exercise when high fat diets are consumed, and Kekwick and Pawan (1957) report higher insensible water loss during a high fat dietary regimen.

The total weight loss (Table 10) in these experiments was no greater than can be adequately explained by glycogen and lean tissue degradation. The lean tissue will be discussed later, but Olsson and Saltin (1970) have shown that 2.4 kg can be gained in weight during glycogen repletion in average young men. The RQ decreased at the end of the high fat periods, indicating a large depletion of muscle glycogen in addition to liver glycogen.

5. Water-balance. Since a high fat diet is reported to produce excessive weight loss by a shift in the water-balance, it was monitored. The total water intake plus metabolic water minus the urine volume, should be equal to sweat loss and insensible water loss, assuming no change in body water and stool water. The water loss is not easy to measure; but if all the factors influencing water-balance (stool volume, insensible evaporation, and sweat) are unaltered, the water-balance should be the same during the carbohydrate and fat diets, and any change represents the extra water loss due to an effect of high fat diets. The environmental temperature and the relative humidity may, however, change--which can be expected to alter the insensible water loss; and there is

some evidence that the insensible water loss is higher on high fat diets. The voluntary activity of the subjects may also vary during the dietary periods; there are reports that a high fat diet decreased activity (Livingston, 1972), and stool volume is affected by diet. However, if the water-balance is unaffected by the diet, the difference in water-balance between the carbohydrate diets and fat diets should correspond to the weight loss during the high fat dietary period (no weight loss during the carbohydrate period), and thus be a measure of the cumulative effect of dehydration and lean tissue catabolism.

From Table 12 (page 115), it is seen that for KB there was a negative mean daily water-balance of 797 g, or a total negative water-balance of almost 4 kg in five days, compared to an actual weight loss of 1.24 kg. This was undoubtedly partly due to differences in weather conditions and differences in stool water. The average water-balance increased 300 ml during the CHO III period (compared to CHO II); if this occurred during the Fat II period it can account for most of the discrepancy. During the Fat II period, the weight loss was 1.14 kg and the difference between the mean water-balance amounts to 1.30 kg (calculated as the difference between the mean water-balance during the previous control period and the high fat period, and multiplied by the five days the experiment lasted).

The metabolic water is ignored in the above calculations, since on a caloric basis, fat and carbohydrate produce nearly the same amount of water. Metabolism of 1 g of carbohydrate produces 0.6 g of water, whereas metabolism of 1 g of fat produces 1.1 g of water. Since the energy requirement of the subject was unchanged, only 4/9 g of fat can

substitute for 1 g of carbohydrate on a caloric basis; under such circumstances, the metabolic water production would be 0.49 g of water per 4 kcal from fat. Since the latter value is not very different from the metabolic water produced by a calorically equivalent amount of carbohydrate (0.6 g per 4 kcal), the difference in metabolic water produced appears negligible in relation to the other factors.

B. Experiment III (Exercise)

1. Standard work. As already pointed out, Experiment I showed more anaerobic oxygen consumption during the high carbohydrate diet. Because of the shorter work and recovery periods (five instead of ten minutes) in this experiment, significant differences in oxygen consumption can be expected. It is seen from Table 15 (page 119) that the oxygen consumption showed much more variability when the subject ran on the treadmill than occurred when the subjects worked on the bicycle ergometer in Experiment I. This was true even when the same diet was consumed. A small variation in the speed of the treadmill has an important effect on the oxygen consumption, whereas the pedaling speed on the bicycle ergometer has no effect on the work performed. The mean oxygen consumptions on the different diets are compared in Table 15, but these experiments are for one subject (KB) only, and with just one or two experiments for each dietary period.

Despite large intra-individual variations, the mean heart rates were equal for the two diets. Because of the low work load, fat was the major substrate during the high fat diets, but a surprisingly high percentage (64%) was from carbohydrate during the high carbohydrate diet.

These experiments therefore, show that under appropriate conditions (exercise of short duration and low intensity), it is possible to promote substrate utilization in the desired direction.

The RQ's need further comment. First, during all the high fat diets, they were very low; but particularly so for Fat II. Such low RQ was also observed by Krogh et al. (1920), but values below 0.69 have generally been ascribed to experimental errors. In the Fat I diet, the fat was primarily from butter and meat, which can partly explain the higher RQ in this experiment, since butterfat has a minimum RQ of 0.72, rather than 0.707 for mixed fats. In the Fat II experiment, corn oil was the primary fat used.

A possible explanation for the low RQ's observed during exercise, during the high fat dietary regimen, is hypoventilation; but since the hydrogen ions associated with ketosis stimulate the respiratory center, that explanation is not a very likely one. Furthermore, hypoventilation would probably not produce consistently low RQ's on the high fat diets.

Hawley et al. (1933) found many studies with consistently low RQ's (that is, below 0.69), comparable to those found when their subjects consumed a high fat diet. They concluded that it could not be due to error, but consistently occurs in some subjects. Such low quotients are also normally found in diabetics. Production of ketone bodies rather than complete oxidation of fat would decrease the RQ's, but for this to become important, ketone bodies would have to accumulate in the blood. If the ketone bodies are produced in the liver and oxidized in the muscle, they have no effect on the RQ. The urinary ketone body

excretion in this experiment was not much different from the excretion in Experiment I, where no such low RQ's were observed.

Hawley et al. (1933) described experiments in which consistently low RQ's (average 0.65) were found in their subjects from 1/2 to three hours after being fed a high protein, high fat meal; furthermore, deliberate underventilation did not change the results. They could find no relation between the degree of ketosis or ketonuria, and the low RQ's. They concluded that the low RQ's were due to the formation of glucose from proteins and stored as glycogen.

Formation of glucose from protein could explain our results obtained in the absorptive state when a high protein, high fat diet was fed. Although the RQ's appear depressed in both fat periods, it is particularly so in the last. As will be discussed later, both BUN and 24-hour urinary nitrogen excretion increased significantly during both fat periods; but particularly during the Fat II diet, which supports the idea of gluconeogenesis taking place from proteins. At higher exercise levels, as during the maximal oxygen consumption determination that followed, the use of muscle glycogen plays a dominating role and no low RQ's were seen.

According to Hultman and Nilsson (1975), glycogen is not stored in either liver or muscle when a high fat diet is fed (at least, only very slowly). But the results of Felig and Wahren (1971a) show that even mild exercise stimulates gluconeogenesis from proteins, which may give the basis for the accumulation of glucose, and possibly explain the low RQ's.

2. Efficiency. From Table 15 (page 119), it is seen that there was no change in the oxygen consumption during recovery; but the mean oxygen consumption in the work period was 17% higher during the high fat periods than during the high carbohydrate periods. When the oxygen consumption is adjusted for the lower caloric value of one liter of oxygen when fat is the substrate (4.6%, assuming pure fat oxidation during the high fat periods), the increase in oxygen consumption is about 12%. This is comparable to the value found by Krogh et al. (1920) and Marsh and Murlin (1928). However, as has just been pointed out, the low RQ's indicate that gluconeogenesis was increased during the exercise period, and this may be the reason for the increased oxygen consumption during exercise in the high fat periods.

Ketosis was similar during the two exercise experiments; but only in the treadmill exercise (Experiment III) was there a decreased efficiency when fat was the major substrate. This indicates that ketosis was not a factor in causing the decreased efficiency. However, these experiments were carried out on one subject only, and with only two experiments during each dietary period. When one also considers the greater uncertainty of determining the exact workload on a treadmill (compared to a bicycle ergometer), these results need further confirmation.

Krogh et al. (1920) found the standard error for the oxygen determinations to be 2% when done on the same day. However, if this is done on a treadmill the error must be considerably larger because variations in the actual speed affect the oxygen consumption (the pedaling speed does not).

3. Maximal oxygen consumption. The RQ's during the determination of the maximal oxygen consumption (Table 16, page 120) are all very high, which is to be expected in high intensity work of short duration, and for the high fat dietary periods. The high RQ's emphasize the importance of muscle glycogen during exercise; however, the RQ during the second week of the Fat II diet (5/26) was somewhat lower, probably indicating a decrease in the glycogen stores. During the experiment on 5/12, which was the second week of the CHO II period, consistently high RQ's were observed, the highest being 1.24. This, of course could be explained by hyperventilation, but since the high RQ's were seen throughout the entire period, and the subject was well acquainted with the procedures, there is no reason to believe that this was the case. Krogh et al. (1920) also occasionally observed such high RQ's, but considered them to be due to hyperventilation. However, another explanation relates to the fact that the subject was in the absorptive state, and during a high carbohydrate diet, this causes fat synthesis from glucose (Owen and Reichard, 1971a). RQ's greater than 1 have also been found by Hatch et al. (1955) in subjects fed the Kempher rice diet. Such high RQ's can best be explained by fat synthesis, which gives a theoretical RQ of 8.0 (Swift and Fisher, 1964).

This experiment shows that there is no difference between the maximal oxygen consumption during the high fat period, and the high carbohydrate periods. During this kind of work, it is likely that the muscle's ability to utilize oxygen is the limiting factor during exercise (Pernow and Saltin, 1971). If this is the case, the maximal oxygen

consumption is a measure of the functioning of the TCA-cycle. And these data then show that the function of the tricarbocylic acid cycle (TCA-cycle) is not decreased during a high fat diet as claimed by Krebs (1964) to be responsible for ketogenesis. This agrees with the hypothesis that ketogenesis is caused by an increased plasma FFA in connection with a decrease in the insulin/glucagon ratio (Cahill, 1976).

IV. SUMMARY

Experiment I did not show any decrease in efficiency when fat was the major substrate; whereas a reduction was seen during Experiment III. Since the increase in ketone body production was similar during the two experiments, this indicates that ketosis is not the cause of the decreased efficiency observed during high fat dietary regimens.

During Experiment III, fat supplied a much higher fraction of the substrate than during Experiment I, probably due to the lower level of exercise, and possibly because the exercise was performed in the absorptive state. The longer duration of the high fat dietary regimen, and presumably a lower muscle glycogen level, may be a factor in lowering the work efficiency in Experiment III. The oxygen consumption was significantly increased during the work period of the second week, as compared to the first week; and the muscle glycogen was probably decreased to low values in the second week. The increased gluconeogenesis could also presumably be a factor in the decreased efficiency. These conclusions are limited by the relatively few experiments with one subject in Experiment III; but they demonstrate that during low work loads of short duration, fat becomes the major energy substrate.

PART TWO: EFFECTS OF THE DIETS

V. EXPERIMENTS II AND III

A. Introduction

As no decrease in efficiency was seen during Experiment I, and less than expected ketosis developed, it appeared likely that the high fat diets (high in polyunsaturated fats and containing soy-protein only) were less ketogenic than conventional high fat diets. Sinclair (1964) postulated that linolenic acid has antiketogenic properties, and this theory appears to be supported by the results of Tantibhedhyangkul et al. (1967), who did not find any ketosis after ingestion of corn oil in the postabsorptive state compared to MCT (medium chain triglycerides).

On this basis it was decided to compare the ketogenic properties of a high fat diet based on dairy cream as used by Christensen (1939), which did produce ketosis, with our high fat diet based on corn oil and soy-protein. The protein content was kept low (5% of calories) in Experiment II.

In Experiment III the protein content was increased to 15% of calories, while the carbohydrate content was unaltered in order to study the effect of protein intake on ketogenesis, gluconeogenesis, and weight loss. Proteins are generally considered antiketogenic (Keit, 1963); however, Worthington and Taylor (1974) found that ketosis increased on a 1200 kcal high protein diet compared to a 1200 kcal mixed diet; and a pemmican diet produced a high degree of ketosis (Mark et al., 1944). Recently Wahren et al. (1976) compared the effect of ingestion of a

protein (beef) meal in diabetic and normal postabsorptive men. In diabetic, but not in normal men, gluconeogenesis increased, showing that under appropriate conditions (low insulin/glucagon ratio), proteins promote gluconeogenesis. In the diabetics (but not in the control group), protein feeding caused a pronounced increase in ketogenesis. The purpose of Experiment III was to evaluate the effect of protein intake on gluconeogenesis, ketogenesis, and dehydration.

B. Procedures

1. Experimental design. The experimental outline can be seen in Table 1 (page 97). Each dietary period lasted five days (beginning Sunday morning and ending Friday morning). But the diet was continued Friday and Saturday until the next diet was introduced Sunday morning (there were no ad lib. periods). The study began with a high carbohydrate control period (CHO I) followed by the Fat I dietary regimen (based on dairy cream). After the CHO II period, the Fat II regimen (based on corn oil and soy-protein) was introduced. The study terminated with a final control period (CHO III).

The 24-hour urine was collected daily, and the subjects were weighted (nude) every morning. The urine was checked for ketones and pH, and the volume recorded. A sample was frozen for analysis of total nitrogen. The subjects recorded their own water intake for calculation of water-balance. A blood sample was taken the last day of each period (at least 12 hours after the last meal), and the second day of the high fat period, for analysis of blood constituents. The subjects continued with their usual physical activity during the

study, and no exercise was carried out except for the standard walk and the maximal oxygen determination with KB (twice during each experimental period in Experiment III).

2. Diets. The calculations of the diets are shown in Appendices E to H, and the approximate composition shown in Table 17. The high carbohydrate control diet (CHO I) was basically the same as the one used during the exercise study (Experiment I). It consisted primarily of fruit, vegetables, cereals, and soy-protein products (supplied free of charge by Worthington Foods, Inc.[1]). The protein content was kept quite low in Experiment II (about 30 g/day) as compared to Experiment III (about 90 g/day). Three different meals were fed each day, but the same menu was repeated throughout each period. The same high carbohydrate control diet was used for all the CHO periods in both experiments.

The Fat I regimen was based on heavy whipping cream (42% fat), the protein content was adjusted with 96% soya protein (from Fearn), and fruit canned in water was added. The cream was whipped, and the other ingredients mixed in a blender before being added to the cream. Sufficient mixture was made for the entire period and frozen. The mixture was fed three times a day for the duration of the period (Fat I).

During the Fat II period, breakfast consisted of soya protein (Fearn), apples, avocado, lemon, and corn oil, mixed in appropriate proportions and eaten with a small amount of granola. Lunch and supper consisted of a few carrots and a piece of soyameat (Worthington, chicken

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[1]Worthington Foods, Inc., 900 Proprietors Rd., Worthington, Ohio 43085.

style) with a dressing made from avocado (Fuerte[1]), corn oil, and pecans. The same menu was repeated every day throughout the period. The composition of the diets is given in Table 17. The caloric intake was adjusted to maintain weight during the first control period (CHO I).

In Experiment II the protein intake was made very low (about 30 g/day) in order to compare the results with those from the higher (90 g/day) protein intake of Experiment III. The daily intake of EAA was calculated and compared with the requirement in Appendices L to N. According to these calculations, the total sulfur-containing AA are lowest, but sufficient, relative to requirement.

TABLE 17. Approximate composition of diets as percent of calories in Experiment II.

Diet	Carbohydrate	Fat	Protein
Control	85	10	5
Fat I[1]	6	89	5
Fat II[2]	6	89	5

[1]Cream.
[2]Corn oil.

3. Analysis. The urine was collected in polyethylene bottles which were kept sealed and in a refrigerator except during collection; pH and volume were determined immediately after the last voiding

[1]Supplied free by the CALIFORNIA AVOCADO SOCIETY, P. O. Box 4816, Saticoy, California 93003.

(in the morning), and a large and a small sample frozen for later use. The urine pH was measured on a Fisher pH/ion meter (model 420) using glass electrodes. The urinary nitrogen was determined using the semi-micro Kjeldahl method. Potassium sulfate was used to increase the boiling point and a 10% copper/sulfate solution was used as catalyst. One-half ml of urine was added to the digestion flask with an Eppendorf pipet (No. 3130). The digest was transferred quantitatively to a 50 ml volumetric flask and analyzed on a technicon autoanalyzer (No. 2) using the color reaction with sodium nitroprusside, the color being read at 660 nm. The relative concentration of acidic compounds in the urine when the subjects ate the various diets was determined by the automated metabolic profiling method (Sweeley et al., 1974). This was done with the LKB 9000 gas chromatograph-mass spectrometer in interphase with a digital PDP 8/c Computer using the MSSMET program as described by Gates (1977). Tropic acid was used as internal standard and analysis was done the last two days of each experimental period.[1]

Blood samples were taken in the postabsorptive state at the Laboratory of Clinical Medicine, Lansing, Michigan, where the serum analyses (automated SMA 12 or SMA 18) were performed using standard procedures (Hycel).[2] The following blood constituents were determined: serum bilirubin, glucose, urea nitrogen, cholesterol, albumin, total protein, uric acid, calcium, phosphorus, and serum glutamic oxaloacetic transaminase

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[1]These analyses were carried out by Stephen Carl Gates, in the laboratory of Professor C. C. Sweeley, of the Biochemistry Department, MSU.

[2]1974 Manual. HYCEL, INC., P. O. Box 36329, Houston, Texas.

(SGOT). The blood was taken on the last day of each dietary period and also the second day of the high fat periods. On the last day of each experimental period, an arterialized blood sample was obtained by immersing the hand in warm water, making a cut in the end of the thumb with a stylet, and filling capillary tubes with blood. The tubes were sealed and used for analysis of pH and pCO_2 on the radiometer (Copenhagen) pH-meter.

Water intakes were recorded by the subjects for calculation of water-balance as described under the exercise study. The body weight was determined in the same way as during the exercise study.

4. Experiment III. This study was similar to Experiment II, except that no metabolic profiling of organic acids by gas chromatography-mass spectrometry was carried out on the urine. Sodium, potassium, chloride, and triglycerides were determined in the blood the last day of each dietary period in addition to the other blood parameters. Urine was collected each day except for weekends, but during CHO I, only during the last week, since the caloric intake was slightly modified after the first week.

The dietary periods were expanded to 14 days for the high carbohydrate control periods, and to 11 days for the high fat study periods; there was no final control period in this study. The first high fat diet (Fat I) consisted primarily of meat, butter, and some cheese and eggs. A few carrots were added. The meat was bought in one lot, cut in suitable pieces, and frozen. On the second high fat diet (Fat II), soy-protein (from Worthington), which contains very little carbohydrate, was

used; the fat came from corn oil and avocadoes (Fuerte). Mayonaise (with polyunsaturated fats) and some cottage cheese were also used. The control diet also contained soy-protein. The composition of the diets is given in Table 18. (The contribution of carbohydrate, fat, and protein was the same in the two high fat diets.) The calculations are shown in Appendices I, J and K.

TABLE 18. Composition of diets as percent of calories in Experiment III.

Diet	Carbohydrate	Fat	Protein
Control	60	25	15
High Fat	5	80	15

The following variables were determined with the Coulter Counter (Model S):[1]

RBC count, million per cubic mm
Hemoglobin (HGB), gms per 100 ml
Hematocrit (HCT), vol. percent
Mean corpuscular volume (MCV): cubic μ
Mean corpuscular hemoglobin (MCH): μμg
Mean corpuscular hemoglobin concentration (MCHC): percent

The subject WH, having kidney problems, did not participate. KB and RH completed this series. A third subject, JG, started this experiment, but found the dietary regimen too unacceptable, and did not complete the study.

[1]These analyses were made by the Laboratory of Clinical Medicine, Lansing, Michigan.

VI. RESULTS FROM EXPERIMENT II

A. General Observations

The subjects generally felt well when consuming the high carbohydrate diet. The stool volume was large and transit time short during the high carbohydrate diet (daily bowel movements); whereas during the high Fat I dietary regimen (dairy cream), the stool was very small and bowel movements very infrequent. During the second high fat diet (Fat II), bowel movement was regular, but the stool volume was small. The subjects generally felt tired or had headache while consuming the high fat diet. On the second day of the first high fat diet, KB was very weak and could hardly walk; but when the blood was checked the next morning at the Laboratory of Clinical Medicine for ketone bodies (by the nitroprusside reaction), none was detected. Apparently, the condition was due to low blood glucose levels. The following morning when the blood samples were taken, the subject felt better. The blood glucose was elevated, probably due to an overshoot in glucose production.

B. Urine Analysis

Urinary pH, total ketone bodies, and total nitrogen excretion are reported in Table 19.

For the last two days of each experimental period, a metabolic profile for organic acids was made on the urine. The amount of the different compounds is expressed relative to the internal standard (tropic acid). In general, the profiles are very similar for the same subjects on the same diet, and there is good agreement in the general trend between the subjects, although the profiles show a pattern

TABLE 19. Urine pH and the excretion of ketones and nitrogen during Experiment II (5% of calories from protein). Each dietary period lasted five days.

		Data for KB			Data for WH		
Date	Diet	pH	Ket.[1]	Total N[2]	pH	Ket.[1]	Total N[2]
1/22	CHO I	7.00		4.86	5.86		5.55
1/23	CHO I	7.17		5.05	7.03		8.45
Mean	CHO I	7.09	0	4.96	6.45	0	7.00
1/25	Fat I	7.23	0	5.83			
1/26	Fat I	5.94	L-M	6.92	5.15	L	9.77
1/27	Fat I	5.62	L-M	10.57	5.28	M	8.34
1/28	Fat I	5.55	L-M	9.77	5.26	M-H	11.34
1/29	Fat I	5.54	M	7.50	5.21	M	10.58
Mean	Fat I	5.98		8.12	5.22		10.01
2/ 5	CHO II	7.40		5.71	6.70		6.14
2/ 6	CHO II	7.10		4.41	6.93		8.82
Mean	CHO II	7.25	0	5.06	6.82	0	7.48
2/ 8	Fat II	6.43	0	11.55	5.97	0	
2/ 9	Fat II	6.42	L-M	9.19	5.60	L-M	12.50
2/10	Fat II	5.37	M	8.80	5.12	M	11.89
2/11	Fat II	5.75	M	7.65	4.82	M	11.64
2/12	Fat II	5.26	M-H	6.24	4.84	M	10.96
Mean	Fat II	5.85		8.69	5.27		11.75
2/18	CHO III	5.97		3.91	6.96		6.80
2/19	CHO III	7.93		3.81			6.92
Mean	CHO III	6.95	0	3.86	6.96	0	6.86

[1]Excretion of ketones in the urine in relation to control levels (zero) graded from low to high (L,M,H).

[2]Nitrogen excretion in the urine (g/day).

characteristic of the individual. Since the three carbohydrate periods are very similar, the average value for these periods is used as a reference to which the mean of the two determinations for each fat diet can be compared. These results are given in Table 20, listing the following compounds for which analysis was made:

LA = Lactic acid

β-OH-Bu = β-hydroxybutyric acid

GL = Glycerol

P = Inorganic phosphorous

Gu = Glutaric acid

Ad = Adipic acid

HMG = β-hydroxy-β-methylglutaric acid

VMA = Vanillyl Mandelic acid

Hx = Hexuronic acid

Hp = Hippuric acid

The metabolic profiling was done according to the method of Gates (1977), which can detect many metabolic excretion products; but in this study, particular attention was given to compounds of special interest in evaluating the effects of the high fat diets, and to compounds that show unusually large variations. Because there are large individual variations in the metabolic profile for most compounds, these data are most useful on an intra-individual basis.

TABLE 20. Urinary excretion of organic acids during Experiment II. (The figures express the peak area relative to that of tropic acid, after the method of metabolic profiling [Gates 1977].)

Compounds[1]	Control[2]	Fat I[2]	Fat II[2]	Control[3]	Fat I[3]	Fat II[3]
LA	.68	.23	.64	1.38	.56	.96
β-OH-Bu	2.41	8.90	9.38	1.83	8.90	8.12
GL	.05	.37	.20	.06	.29	.16
P	.89	.70	.82	1.04	.46	.25
Gu	.73	.70	.60	.66	.08	.17
Ad	.16	2.57	.88	.07	.46	.10
HMG	2.00	.38	.25	.32	.08	.10
Ci	.30	.33	.52	.28	.11	.20
VMA	5.21	.35	.72	1.12	.27	.26
Hx	2.38	2.00	1.57	1.01	.41	.30
Hp	10.00	2.75	6.88	6.48	.39	1.26

[1]For explanation of these abbreviations, see page 142.

[2]Values for KB.

[3]Values for WH.

C. Blood Analysis

On the last day of each period, an arterialized blood sample was taken and analyzed for pH and pCO_2. The results are given in Table 21.[1] (The pCO_2 data are not complete.)

TABLE 21. Arterialized pH and pCO_2 as mm Hg in Experiment II.

Diet	pH[1]	pCO_2[1]	pH[2]	pCO_2[2]
Control	7.43		7.40	
Fat I	7.41		7.37	
Control	7.45	34.4	7.42	38.0
Fat II	7.41	34.9	7.37	33.8
Control	7.43		7.40	37.0

[1]Values for KB.
[2]Values for WH.

The results of the blood analysis are shown in Table 22. The abbreviations, units, and adult normal ranges are given below:

Chol = Cholesterol, 150-300 mg/dl

Ca = Calcium, 8.5-10.5 mg/dl

P = Phosphorus, 2.5-4.5 mg/dl

Bili = Bilirubin, 0.2-1.5 mg/dl

[1]These pH values were measured by Gary R. Hunter in the Laboratory of Professor Richard R. Heisey, Department of Physiology, MSU.

Alb = Albumin, 3.5-5.0 g/dl

TP = Total protein, 6.0-8.0 g/dl

UA = Uric acid, up to 8.5 mg/dl (for males)

BUN = Blood urea nitrogen, 6-23 mg/dl

Glu = Glucose, 70-115 mg/dl

SGOT = Serum glutamic oxaloacetic transaminase, 2-45 μ/L

Na = Sodium, 135-145 mEq/L

K = Potassium, 3.3-4.6 mEq/L

Cl = Chloride, 96-107 mEq/L

TG = Triglycerides, 50-155 mg/dl

The cholesterol increase both on the saturated and unsaturated high fat diets, while total serum proteins, indicate some dehydration as will be discussed in the section evaluation of data.

D. Weight and Water-balance

The body weight, total water intake (TWI)(including water in the food), urine volume (UV), and water-balance (WB) (calculated as TWI-UV), are recorded in Table 23. The urine volume shows great variation during the high fat regimens, which may be due to variation in glycogen and lean tissue catebolism.

VII. RESULTS FROM EXPERIMENT III

A. Urine Analysis

The pH was measured in the urine as before, and total nitrogen and ketone bodies determined. The results are given in Table 24.

TABLE 22.[1] Blood constituents during Experiment II (5% of calories from proteins).

Date	Diet[2]	Chol	Ca	P	Bili	Alb	TP	UA	BUN	Glu	SGOT
1/23[3]	Control	181	10.2	1.6	.6	4.5	6.5	5.9	10.2	88	29
1/26	Fat I	178	9.9	3.1	.6	4.6	6.8	6.6	18.0	112	37
1/30	Fat I	230	10.3	2.8	.7	4.9	7.3	9.5	19.5	68	67
2/ 6	Control	184	9.9	2.5	.5	4.5	6.4	5.2	9.3	88	32
2/10	Fat II	219	10.2	3.3	.8	4.5	6.9	6.4	17.1	62	39
2/13	Fat II	225	10.5	2.9	.8	4.9	6.8	7.9	12.7	76	40
1/20	Control	195	9.9	2.2	.5	4.1	6.2	5.0	10.0	77	25
1/23[4]	Control	205	9.8	2.9	.8	4.6	7.2	8.4	10.3	92	48
1/30	Fat I	210	10.3	3.4	.9	5.0	7.8	15.9	13.9	102	93
2/ 6	Control	194	10.3	2.9	.8	4.6	7.0	7.7	9.8	102	43
2/10	Fat II	192	9.9	3.5	.7	4.8	7.3	8.2	13.2	92	52
2/13	Fat II	204	9.8	3.0	.7	4.8	7.8	12.4	10.8	75	48
2/20	Control	220	9.9	3.6	.6	4.8	7.2	6.7	11.5	86	33

[1]For explanation of abbreviations, see pages 144-145.

[2]Fat I--fat from dairy cream. Fat II--fat from corn oil.

[3]Results for KB.

[4]Results for WH.

TABLE 23. Water-balance and body weight data during Experiment II.

Date	Diet[1]	Data for KB				Data for WH			
		TWI[2]	UV[3]	WB[4]	Wt.[5]	TWI[2]	UV[3]	WB[4]	Wt.[5]
1/22	CHO I	2210	600	1610	64.1	2860	670	2190	104.5
1/23	CHO I	2060	650	1410	63.6	3440	1126	2214	104.5
1/25	Fat I	1025	1330	70	64.1	2265			104.5
1/26	Fat I	1025	570	825	62.5	2385	1350	1035	102.7
1/27	Fat I	1225	940	655	61.4	2545	880	1665	102.5
1/28	Fat I	1225	1240	255	60.9	2715	1610	1105	101.8
1/29	Fat I	825	610	585	60.5	2720	1620	1100	99.1
2/ 4	CHO II	2060	900	1160	62.3	3620	1400	2220	104.1
2/ 5	CHO II	2020	820	1200	63.0	3620	1200	2420	104.5
2/ 8	Fat II	1645	1500	145	63.2	2345	1640	705	104.5
2/ 9	Fat II	2345	2060	285	62.5	2335	1510	725	104.1
2/10	Fat II	1645	1000	645	61.6	2685	1660	1025	103.2
2/11	Fat II	1390	850	540	61.1	4145	2970	1175	102.3
2/12	Fat II	1645	650	995	61.1	2665	1670	995	99.1
2/18	CHO III	1810	650	1160	62.0	3360	1240	2120	101.4
2/19	CHO III	1810	650	1160	62.3	2540	850	1690	103.6

[1]CHO = high carbohydrate control diet.
Fat I = high fat diet based on dairy cream.
Fat II = high fat diet based on corn oil and avocado.

[2]Total water intake in ml per 24 hrs.

[3]Urine volume in ml per 24 hrs.

[4]Water-balance = TWI-UV.

[5]Body weight of the subjects in kg.

TABLE 24. The pH, ketones and nitrogen excretion in the urine during Experiment III (15% of calories from proteins).

Date	Diet[1]	Data for KB			Data for RH		
		pH	Ketones[2]	UN[3]	pH	Ketones[2]	UN[3]
4/11	CHO I	6.76	none	10.59	6.98	none	
4/12	CHO I	6.85	none	12.13	6.85	none	11.21
4/13	CHO I	6.87	none	9.22	6.56	none	10.71
4/14	CHO I	6.86	none	11.29	6.78	none	8.89
4/15	CHO I	7.08	none	11.84	7.15	none	10.16
Mean	CHO I	6.88	0	11.01	6.86	0	10.24
4/18	Fat I	6.68	Tr.	12.35	6.30	Tr.	13.66
4/19	Fat I	5.55	M	15.65	5.80	M	14.06
4/20	Fat I	5.42	M	17.79	5.64	M	15.07
4/21	Fat I	5.47	M	14.01	5.72	M	18.37
4/22	Fat I	5.47	M	14.14	5.90	L	12.00
4/25	Fat I	5.65	M	12.13	5.92	M	13.61
4/26	Fat I	5.67	L	11.76	5.61	L	16.66
4/27	Fat I	5.64	L	14.76	5.46	M	15.60
4/28	Fat I	5.74	L	11.21	5.72	M	12.56
Mean	Fat I	5.70		13.76	5.79		14.62
4/29	CHO II	5.91	none	13.01	5.42	none	13.74
4/30	CHO II	6.61	none	11.34	6.22	none	10.33
5/ 1	CHO II	6.16	none	10.51	6.41	none	10.97
5/ 2	CHO II	7.13	none	11.57	6.64	none	11.28
5/ 3	CHO II	6.80	none	9.31	6.76	none	11.59
5/ 4	CHO II	6.85	none	9.52	6.53	none	10.98
5/ 5	CHO II	7.08	none	9.87	6.50	none	7.95
5/ 6	CHO II	6.86	none	10.30	6.61	none	8.61
5/ 9	CHO II	7.00	none	9.62	6.54	none	9.14
5/10	CHO II	6.86	none	9.69	6.71	none	12.83
5/11	CHO II	6.75	none	10.10	6.99	none	11.28
5/12	CHO II	6.65	none	11.07	6.53	none	13.11
5/13	CHO II	6.74	none	10.66	6.58	none	8.50
Mean	CHO II	6.72	0	10.51	6.50	0	10.79

continued

TABLE 24--continued

Date	Diet[1]	Data for KB			Data for RH		
		pH	Ketones[2]	UN[3]	pH	Ketones[2]	UN[3]
5/17	Fat II	6.85	0	13.20	6.60	0	13.52
5/18	Fat II	5.85	L	14.46	5.68	L	14.74
5/19	Fat II	6.00	M	20.01	6.30	L-M	14.40
5/20	Fat II	5.51	L-M	16.92	5.65	L	13.81
5/23	Fat II	5.49	H	16.87	5.90	L	14.31
5/24	Fat II	5.71	H	17.82	5.86	L	15.50
5/25	Fat II	5.85	M	19.25	6.50	M	14.71
5/26	Fat II	5.79	H	14.30	5.68	M	16.74
5/27	Fat II	5.74	H	17.20	5.92	H	18.17
Mean	Fat II	5.87		16.67	6.01		15.10

[1]CHO I and II --high carbohydrate, control diet.
Fat I--fat from meat and butter.
Fat II--fat from corn oil.

[2]Levels above control values, listed as Trace through High.

[3]Total nitrogen excretion in the urine (g/day). The high UN values during the high fat periods are due to endogenous protein catabolism.

Since the blood sample was taken in the morning, the BUN probably best corresponds to the nitrogen excreted in the urine on the previous day.

B. Blood Analysis

Blood samples were taken in the postabsorptive state on the fifth and twelfth day of CHO I; on the second, fifth and eleventh day of Fat I; on the third, seventh and fourteen day of CHO II; and on the second, seventh and eleventh day of Fat II. The results are reported in Table 25, in addition to blood constituents, hematocrit and hemoglobin data. RBC, HGB, HCT, MCV, MCH, and MCHC are reported in Table 26.

C. Body Weight and Water-Balance

As in the first study, total water intake (TWI), and urine volume (UV) were measured, and the difference between TWI and UV (WB) was calculated to secure an indication of the water-balance (WB). The results are given in Table 27, together with the body weight in kg. There is some indication of a slight dehydration during the Fat I dietary regimen, based on meat and butter, but due to the variation in the data (RBC and MCH), it may not be significant.

VIII. EVALUATION OF DATA

A. Ketosis

The level of ketones in the urine was evaluated, based on the color reaction with nitroprusside, and by gas chromatography where the peak area of β-hydroxybutyrate was compared to the peak area of the internal standard (tropic acid) in the metabolic profiling of organic acids in the urine.

TABLE 25.[1] Blood constituents during Experiment III (15% of calories from proteins).

Date	Diet[2]	Chol	Ca	P	Bili	Alb	TP	US	BUN	Glu	SGOT
4/ 9[3]	CHO I	212	9.7	2.7	.7	4.0	6.5	5.2	14.2	83.4	23.2
4/16	CHO I	212	9.6	2.9	.8	4.0	6.4	5.4	15.7	79.9	20.3
4/20	Fat I	245	9.7	3.0	.8	4.1	6.6	6.6	20.3	64.4	32.9
4/23	Fat I	269	9.8	3.3	.8	4.0	6.7	7.4	18.3	64.3	30.5
4/29	Fat I	292	10.1	3.3	.6	3.9	6.7	6.1	16.1	76.6	21.6
5/ 3	CHO II	256	9.6	2.9	.4	3.6	6.4	4.2	10.1	77.2	18.6
5/ 7	CHO II	232	9.5	2.9	.6	3.8	6.2	5.1	12.6	88.3	20.2
5/14	CHO II	234	9.5	2.7	.7	4.5	6.6	5.2	15.2	83.0	24.0
5/19	Fat II	230	9.6	2.8	.8	3.6	6.5	5.0	12.1	73.7	26.7
5/24	Fat II	198	9.2	2.8	.6	4.7	6.3	5.3	19.8	79.2	32.4
5/28	Fat II	186	9.3	2.9	.6	3.9	6.0	5.3	27.9	71.5	26.9
4/ 9[4]	CHO I	163	9.5	3.1	.8	4.1	7.2	5.4	9.7	94.7	21.6
4/16	CHO I	156	9.4	3.6	.7	3.9	6.6	4.8	10.9	84.0	22.0
4/20	Fat I	191	9.4	3.6	.9	4.6	7.3	6.6	13.7	80.1	29.6
4/23	Fat I	186	9.5	4.0	.6	4.1	7.2	5.9	10.0	89.4	23.1
4/29	Fat I	214	9.6	3.7	.5	4.0	7.2	5.9	10.8	81.8	26.3
5/ 3	CHO II	176	9.4	3.5	.7	3.9	6.9	4.4	8.6	93.5	19.5
5/ 7	CHO II	174	9.4	3.4	.8	4.0	6.9	4.2	13.2	96.3	21.9
5/14	CHO II	159	9.1	3.5	.5	4.1	7.1	4.7	12.2	89.6	30.2
5/19	Fat II	140	9.3	3.2	.7	3.8	6.9	5.0	11.6	91.3	24.9
5/24	Fat II	129	9.0	3.4	.7	4.6	6.8	4.7	13.5	89.7	26.1
5/28	Fat II	144	9.2	3.3	.9	4.6	7.2	5.2	17.2	72.3	30.1

continued

TABLE 25--continued

Date	Diet[2]	Na	K	Cl	TG
4/29[3]	Fat I	144.7	4.2	104.2	82.6
5/14	CHO II	141.4	4.1	102.4	73.2
5/28	Fat II	142.3	4.0	102.9	98.2
5/29[4]	Fat I	145.3	4.3	105.5	76.3
5/14	CHO II	139.4	4.3	102.5	83.5
5/28	Fat II	141.1	3.8	101.6	73.0

[1]For explanation of abbreviations, normal ranges and units, see pages 144-145.

[2]CHO I and II is the control--high carbohydrate diet; Fat I--fat from meat and butter; Fat II--fat from corn oil.

[3]Results for KB.

[4]Results for RH.

TABLE 26. Hemoglobin and hematocrit during Experiment III.

Date	Data for KB						Data for RH					
	RBC[1]	HGB[2]	HCT[3]	MCV[4]	MCH[5]	MCHC[6]	RBC[1]	HGB[2]	HCT[3]	MCV[4]	MCH[5]	MCHC[6]
4/ 9[7]	4.86	13.9	42	86	28	33	5.08	14.5	44	86	29	33
4/16	4.85	13.7	41	85	28	33	4.93	14.1	42	85	29	33
4/20[8]	5.16	14.5	44	85	28	33	5.16	14.9	45	86	29	33
4/23	4.96	14.5	42	84	29	35	5.05	15.0	44	86	30	35
4/29	4.91	14.3	41	83	29	35	5.08	15.1	44	85	30	35
5/ 3[7]	4.76	13.6	41	85	29	34	4.99	14.5	43	86	29	34
5/ 7	4.59	13.3	39	85	29	34	5.06	15.0	44	86	30	34
5/14	4.85	14.1	41	85	29	34	4.87	14.6	42	86	30	35
5/19[8]	4.74	13.9	40	85	29	35	4.74	14.2	41	87	30	35
5/24	4.74	13.3	39	82	29	35	5.08	14.9	42	83	30	35
5/28	4.57	13.3	38	84	29	35	5.10	15.2	44	86	30	35

[1]Red Blood Cell Count (million/cubic mm)

[2]Hemoglobin (g)

[3]Hematocrit (percent)

[4]Mean corpuscular volume (cubic μ/corpuscle)

[5]Mean corpuscular hemoglobin concentration (μμg/corpuscle)

[6]Mean corpuscular hemoglobin concentration (percent)

[7]High carbohydrate, control diet

[8]High fat diets

TABLE 27. Water-balance and body weight data during Experiment III.

Date	Diet[1]	Data for KB				Data for RH			
		TWI[2]	UV[3]	KB[4]	Weight[5]	TWI[2]	UV[3]	WB[4]	Weight[5]
4/11	CHO I	2346	830	1516	63.6	2828	1430	1398	60.7
4/12	CHO I	2176	940	1236	64.1	3169	2100	1069	60.5
4/13	CHO I	2630	990	1640	63.6	3254	2100	1154	60.5
4/14	CHO I	2517	875	1642	63.6	2800	1380	1420	60.5
4/15	CHO I	2517	950	1567	64.3	2828	1010	1818	60.5
4/18	Fat I	1434	950	484	64.3	2553	2095	458	61.1
4/19	Fat I	1944	1050	894	64.1	2071	1635	436	60.5
4/20	Fat I	2341	1550	791	63.4	1788	1350	438	60.5
4/21	Fat I	1263	1330	133	62.7	2213	1120	1093	59.5
4/22	Fat I	2227	1040	1187	62.3	2185	2000	185	59.5
4/25	Fat I	1037	730	307	61.4	1930	1620	310	59.1
4/26	Fat I	1377	700	677	61.4	1759	1735	24	59.1
4/27	Fat I	1490	1190	300	61.4	2241	1200	1041	59.1
4/28	Fat I	1604	950	654	61.4	1986	1570	416	59.1
4/29	CHO II	2687	770	1917	61.4	3282	1360	1922	58.6
4/30	CHO II	2233	780	1453	62.3	2800	1230	1570	59.5
5/ 1	CHO II	2460	740	1720	63.2	2885	1455	1430	59.5
5/ 2	CHO II	1893	1195	698	63.6	3282	2330	952	59.8
5/ 3	CHO II	2403	950	1453	63.6	2857	1665	1202	59.8
5/ 4	CHO II	2233	1005	1228	64.1	3084	1960	1124	60.5
5/ 5	CHO II	2233	1030	1203	64.1	2687	960	1727	60.5
5/ 6	CHO II	2120	1020	1100	64.1	2573	1170	1403	60.5
5/ 9	CHO II	2006	830	1176	64.5	3254	1450	1804	60.9
5/10	CHO II	2573	850	1723	64.5	2715	1080	1635	60.5
5/12	CHO II	2630	940	1690	64.5	3310	1630	1680	60.7
5/13	CHO II	2516	860	1710	64.1	2176	1090	1086	60.7
5/17	Fat II	2176	1650	517	64.5	2451	1040	1411	60.2
5/18	Fat II	2167	1130	1037	64.1	2734	1819	915	60.0
5/19	Fat II	1770	1220	550	63.6	3310	2045	1265	59.5
5/20	Fat II	1714	940	774	63.2	2933	1020	1913	59.5
5/23	Fat II	1997	930	1067	62.6	2593	1350	1242	59.5
5/24	Fat II	1997	1000	997	63.6	3010	1520	1498	58.9
5/25	Fat II	1770	1100	670	63.2	2479	1420	1079	58.9
5/26	Fat II	1884	855	1029	62.7	3160	1610	1550	58.6
5/27	Fat II	1657	860	797	62.7	3500	1540	1960	58.6

[1]CHO (I and II) is high carbohydrate control diet, Fat I is the high fat diet based on butter and meat[1], and Fat II is based on corn oil and avocados.

[2]The total water intake includes the water contained in the food.

[3]24-hr urine volume (ml).

[4]Water-balance = TWI-UV.

[5]Body weight of the subjects in kg.

The data in Table 19 (page 141) show that increased ketone levels (above resting levels) began to appear in the urine the second day of the high fat diet, and steadily increased throughout the period, although some indication of stabilization of the ketone level is seen. There does not appear to be any difference between the two types of fat diets or between the subjects.

From the data in Table 20 (page 143), there is seen a difference in the control level of ketones (the mean of the three control periods) between the subjects; but not much difference in the final level of β-hydroxybutyric acid during the high fat periods. For KB, the relative value increased from 2.41 to 9.38, about a four-fold increase. For WH, the relative value increased from 1.83 to 8.12, about a 4.5-fold increase. WH, however, reached the highest value of 8.9 on the fourth day of the dietary period; whereas, KB reached the highest value on the last (5th) day of the period.

When we look at the ketone production as determined by the nitroprusside reaction during Experiment III (with high levels of protein, Table 24 page 148), there again is no important difference in ketone production between the two diets or between the subjects. Large day-to-day variations are seen, which may be due to variation in the activity level of the subjects. There is a tendency toward a lower level of ketosis in this study compared to Experiment II, but the day-to-day variation makes it difficult to compare the results. The fat level in Experiment III was lower than in Experiment II, but that was probably not important, since the carbohydrate intake was the same and it is the

deficiency of carbohydrate rather than an increase in fat intake that causes ketone production (Weis and Löffler, 1970). The higher intake of proteins can, however, be expected to decrease ketosis, as has been reported by Bell et al. (1969).

The gradual increase in ketone production seen in these experiments is in good agreement with the data of Bloom (1967); Bell et al. (1969); and Cahill (1976). It seems reasonable to conclude that there is no difference in the degree of ketosis produced by the two types of fat in these diets. Although ketosis is primarily determined by the level of carbohydrate in the diet, the level of protein may have a slight effect.

B. Urinary Nitrogen Excretion, BUN, and Blood Glucose

The effect of the different dietary regimens can be seen from Tables 19, 22, and 24 (pages 141, 143, and 148, respectively). The mean urinary nitrogen excretions (MUN) for the different dietary periods are given in Table 28; also included for comparison are mean BUN (MBUN), mean blood glucose (MGlu), and mean SGOT (MSGOT).

It is clear that the mean values given in Table 28 do not reflect the variation throughout a period, as can be verified by comparing the data in that table with those in Tables 19, 22, and 24. For example, blood glucose tends to be above control values immediately after the high fat diet is introduced, when both glycogenolysis and gluconeogenesis are actively supporting the blood glucose level; but when the liver glycogen is depleted, and gluconeogenesis alone supports the blood glucose level, very low levels are often seen (Hultman et al., 1974).

TABLE 28.[1] Mean values of BUN, blood glucose, serum SGOT, and the nitrogen excretion in the urine during Experiments II and III.

Diet	MUN[3]	MBUN[4]	MGlu[5]	MSGOT[6]	MUN[3]	MBUN[4]	Mglu[5]	MSGOT[6]
	Experiment II (5% of calories from protein)							
Subject[2]		KB				WH		
CHO I	4.96	10.2	88	29	7.00	10.3	92	48
Fat I	8.12	18.8	90	52	10.01	13.9	102	93
CHO II	5.06	9.3	88	32	7.48	9.8	102	43
Fat II	8.69	14.9	69	40	11.75	12.0	84	50
CHO III	3.86	10.0	77	25	6.86	11.5	86	33
	Experiment III (15% of calories from protein)							
Subject[2]		KB				RH		
Control	11.01	15.0	81.7	21.8	10.24	10.3	89.4	21.8
Fat I	13.73	18.2	68.4	31.7	14.62	11.5	83.8	26.3
Control	10.51	12.6	82.8	20.9	10.79	11.3	93.1	23.9
Fat II	16.67	22.9	74.8	28.7	15.10	14.1	84.4	27.0

[1]The caloric intakes were: KB, 2400 kcal; WH, 2700 kcal; and RH, 2300 kcal.

[2]Weight of subjects: KB = 140 lbs; WH = 230 lbs; RH = 135 lbs.

[3]The mean nitrogen excretion for each dietary period (g/day).

[4]The mean BUN for each dietary period (mg/dl).

[5]The mean blood glucose concentration for each dietary period (mg/dl).

[6]The mean SGOT level for each dietary period (μ/L).

Likewise, systematic changes are seen in the urinary nitrogen excretion during the high fat periods, which are probably due to metabolic (hormonal) adaptation to the prolonged exposure to a low protein diet as described by Cahill (1976). It should be emphasized that the protein intake is constant throughout each experiment.

Despite such shortcomings, the mean values are useful in evaluating the results. The urinary nitrogen excretion during the control period for Experiment II is higher than expected on the basis of the protein intake, and may indicate a negative nitrogen balance. However, only two measurements were taken for the control period, so the values may be too high. The urinary nitrogen excretion for the high protein diet (Experiment III) agrees well with the protein intake.

The urinary nitrogen excretion during the fat periods increased about 4 g compared to the control, regardless of whether the nitrogen intake was 5% or 15% of the calories (Table 28). This means that about 25 g of endogenous protein were used for gluconeogenesis. The corresponding BUN was elevated (particularly in KB) during Experiment II (up to 100%), but only increased to a minor extent in the other subjects (Tables 22 and 25, pages 146 and 151, respectively). The BUN increased to about 20 mg/dl when KB consumed the high fat diet, regardless of the protein intake. The only exception to this was the Fat II diet in Experiment II (Table 22), where the BUN was almost back to normal the last day of the period. The reason for this may be found in the steadily declining urinary nitrogen excretion during the period, as seen from Table 19 (page 141). At that time the subjects had had a low protein

intake for four weeks; and KB was probably beginning to adjust to it. The long-term protein sparing effect may be comparable to that discussed by Cahill (1976).

When the mean BUN's for the two protein levels are compared for KB during the control periods, it is seen that a tripling of the protein intake increased the BUN value about one-third, which is in good agreement with the results of Addis et al. (1947) who reported much higher absolute values. The difference may be due to the fact that their blood samples were taken about four hours after lunch; whereas, our results were secured with blood drawn after an overnight fast.

Generally, the blood glucose level fell during the high fat diets; but as long as liver glycogen was available for glycogenolysis, blood glucose levels appeared to be elevated. When the liver glycogen is depleted, the blood glucose level falls, often to hypoglycemic levels, indicating that gluconeogenesis cannot keep up with glucose utilization. As the subjects continued to consume the low carbohydrate diet (high fat), ketone production increased, allowing the blood glucose levels to return toward normal (because of its glucose sparing effect). This probably explains the improved well-being reported by the subjects after the first few days of the high fat diets. These results agree well with the findings of Hultman et al. (1974); and Cahill (1976). The subjective feelings of weakness and fatigue were always most severe on the second or third day, corresponding to the low blood glucose levels before ketone production became maximum.

According to White et al. (1973a), the amino-transferases increase during fasting to facilitate gluconeogenesis from amino acids (AA). As seen from Table 28 the values of SGOT in the blood when the subjects were consuming the high fat diets increased, compared to the control periods. This again indicates that gluconeogenesis from AA takes place during carbohydrate deprivation, but it can also be an artifact due to hemolysis of RBC (which was occasionally seen during the high fat studies).

The relationship between BUN, urinary nitrogen excretion, and blood glucose is plotted for KB in Figure 2 for Experiment II (low protain intake), and in Figure 3 for Experiment III (high protein intake). The data for RH during Experiment III are plotted in Figure 4.

Looking at Figures 2 and 3, we see a fairly good positive correlation between the urinary nitrogen excretion and the BUN; however, when the high carbohydrate control diets were consumed, the BUN levels were lower than would be expected from the urinary nitrogen excretion, indicating that factors other than nitrogen excretion affect the BUN level. Similar results are seen in Figure 4 for RH in Experiment III, although the BUN's were not quite so high during the high fat periods. A decrease in BUN when wheat protein was fed, was observed by Bolourchi et al. (1968).

In most cases the first blood sample was taken 48 hours (or more) after introduction of the high fat diet. At that time, all the liver glycogen should have been depleted. According to Hultman et al. (1974) no compensatory increase in gluconeogenesis takes place under resting

FIGURE 2. Nitrogen excretion in the urine, BUN, and blood glucose for KB during Experiment II. (Fat I, from dairy cream; Fat II, from corn oil and avocadoes with 5% of calories from proteins.)

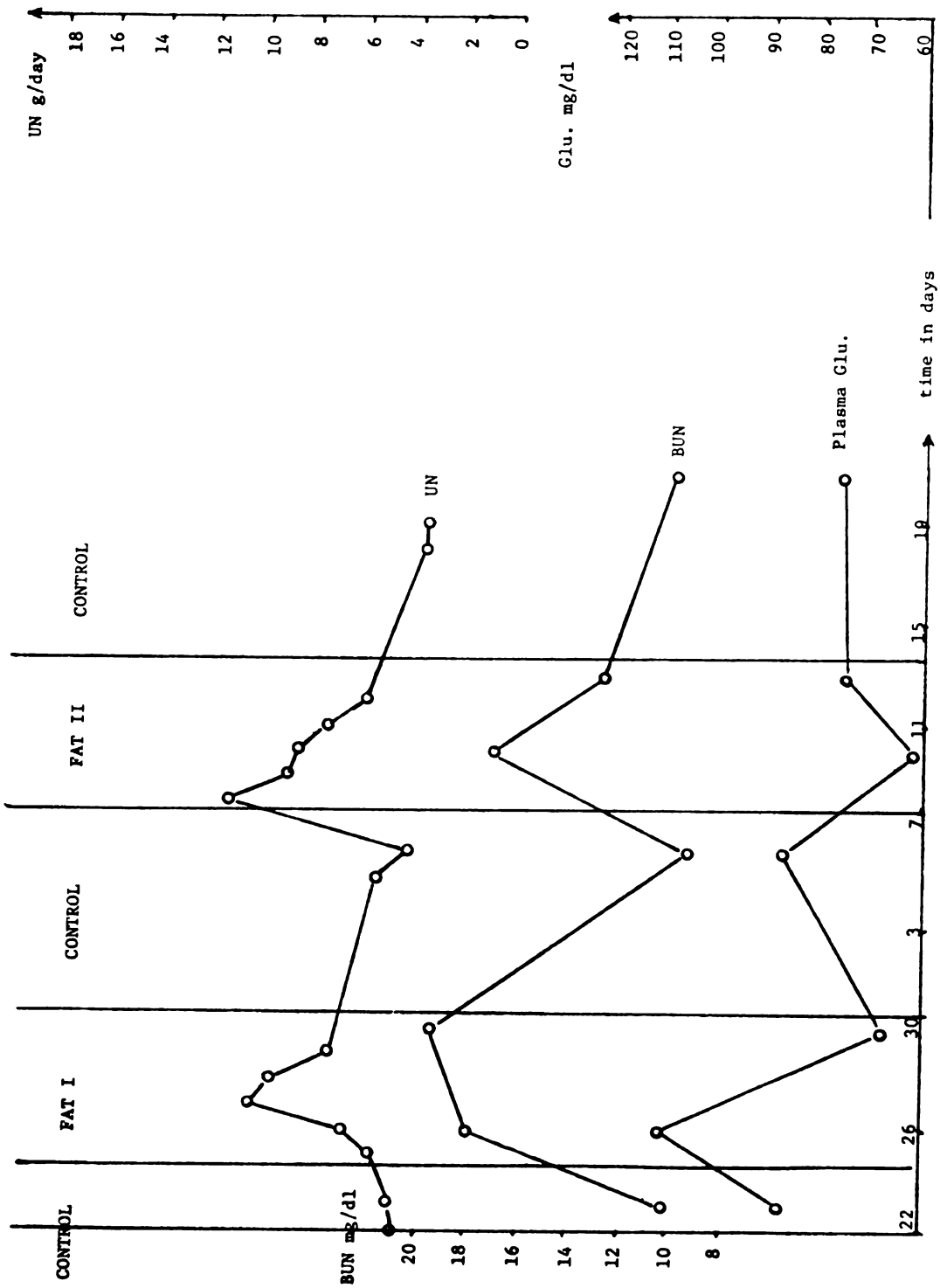
UN g/day
18
16
14
12
10
8
6
4
2
0
Glu. mg/dl
120
110
100
90
80
70
60
CONTROL
FAT II
CONTROL
FAT I
CONTROL
UN
BUN
Plasma Glu.
BUN mg/dl
20
18
16
14
12
10
8
22
26
30
3
7
11
15
18
time in days

FIGURE 2

FIGURE 3. Nitrogen excretion in the urine, BUN, and blood glucose for KB during Experiment III. (Fat I, from butter and meat; Fat II, from corn oil and avocadoes with 15% of the calories from proteins.)

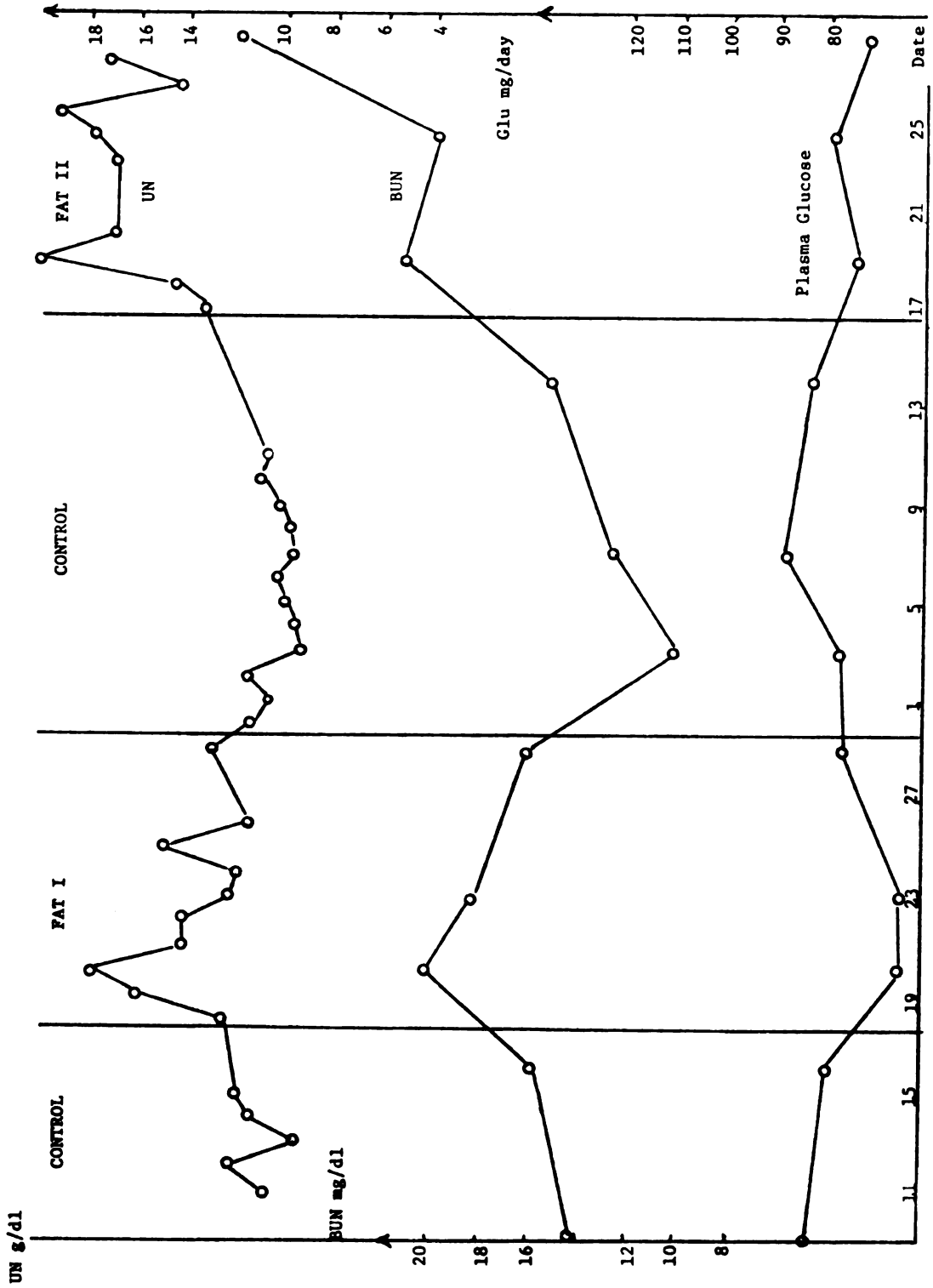
UN g/dl
CONTROL
FAT I
CONTROL
FAT II
UN
BUN
Glu mg/day
Plasma Glucose
BUN mg/dl
Date
11
15
19
23
27
1
5
9
13
17
21
25
18
16
14
10
8
6
4
120
110
100
90
80
20
18
16
12
10
8

FIGURE 3

FIGURE 4. Nitrogen excretion in the urine, BUN, and blood glucose for RH during Experiment III. (Fat I, from butter and meat; Fat II, from corn oil and avocadoes with 15% of calories from proteins.)

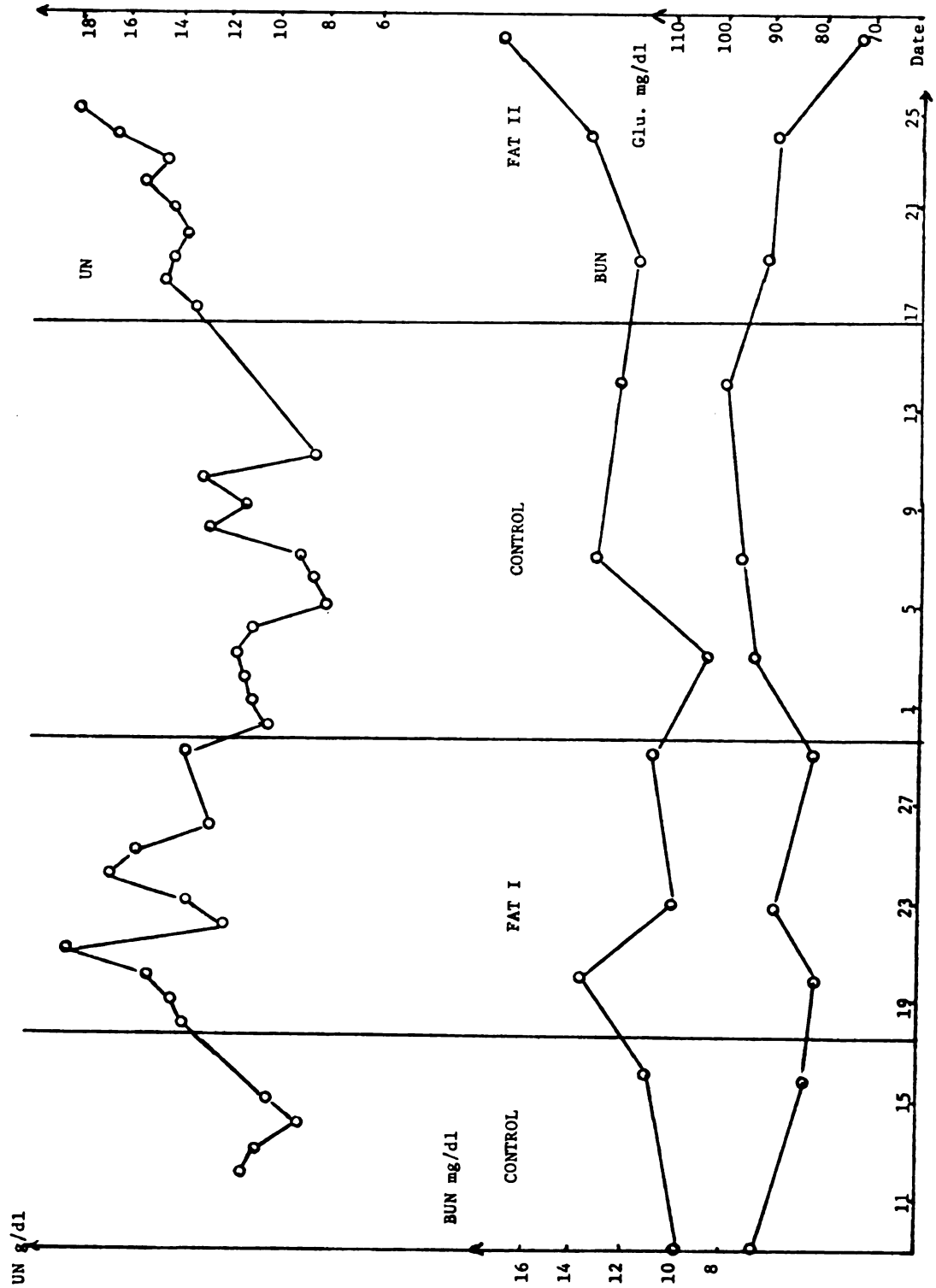
UN g/dl
BUN mg/dl
Glu. mg/dl
Date
UN
BUN
CONTROL
FAT I
CONTROL
FAT II
18
16
14
12
10
8
6
110
100
90
80
70
11
15
19
23
27
1
5
9
13
17
21
25

FIGURE 4

conditions, which explains the low blood glucose level seen at this time. As carbohydrate deprivation continues, the increased ketone production decreases the utilization of glucose, enabling gluconeogenesis to maintain the blood glucose level (Cahill, 1976; and Hultman et al., 1974). During the Fat II regimen (polyunsaturated fat and soy-protein) in Experiment III, no such adjustment is seen. Both the urinary ketone bodies and the urinary nitrogen continued at high or increasing levels, while blood glucose remained low or even decreased. The reason for this is not known.

During the Fat I period in Experiment II, the first blood sample was taken 24 hours after the dietary period began, which explains the high blood glucose (112 mg/dl) seen in KB (Figure 2). Apparently, the combined effect of gluconeogenesis and glycogenolysis produced an overshoot in the blood glucose level. The blood glucose level during the high fat diets was sufficiently low, so that if challenged with exercise, the blood glucose level could severely limit performance. However, under such circumstances, gluconeogenesis from protein is further increased (Felig, 1975).

In most cases, gluconeogenesis (as measured by the increase in urinary nitrogen excretion and BUN) reached a peak early in the high fat dietary periods and then gradually decreased toward the end (Figures 2, 3, and 4). This agrees well with the results of Pozefsky et al. (1976), who found a decreased serum alanine, and decreased alanine release from the forearm muscle during a 60-hour fast compared to a 24-hour fast. The decreased release of gluconeogenic AA during prolonged

fasting is believed to be due to increased levels of branched chain AA (Cahill, 1976). For some reason, such a decrease in gluconeogenesis was not seen during the Fat II regimen (corn oil, soy-protein) in Experiment III; whereas, it was observed on the same type of fat diet with a low protein intake (Experiment II). Though the reason for this is not known, it could, presumably, be due to either an altered AA profile caused by the source of protein (soy-protein), or an altered hormonal response.

The linear regression lines and correlation coefficients (r) between UN and BUN, and between UN and Glu (in plasma) during Experiment II, were calculated for KB; for Experiment II:

BUN = 1.72 UN + 3.24; r = 0.71

Glu = -4.78 UN + 111.0; r = -0.51

For KB during Experiment III:

BUN = 1.16 UN + 1.81; r = 0.75

Glu = -1.20 UN + 92.7; r = -0.50

C. Other Blood Constituents

The mean values for calcium, inorganic phosphate, bilirubin and uric acid during the different dietary periods are given in Table 29. There were no important differences in blood calcium level, which is not surprising in view of the strict homeostatic regulation of this ion. During Experiment II, there was a tendency for the inorganic phosphate concentration to be higher during the high fat diets. However, in Experiment III, this was the case only when the protein came from meat. As seen from Table 21 (page 144), the pH of arterialized blood decreased

TABLE 29. Serum calcium, phosphorous, bilirubin, and uric acid (in mg/dl).

Diet	Serum Calcium		Phosphorous		Bilirubin		Uric Acid	
			Experiment II					
Control	10.2[1]	9.8[2]	1.6[1]	2.9[2]	.6[1]	.8[2]	5.9[1]	8.4[2]
Fat I[4]	10.1	10.3	3.0	3.4	.7	.9	8.1	15.9
Control	9.9	10.3	2.5	2.9	.5	.8	5.2	7.7
Fat II[5]	10.4	9.9	3.1	3.3	.8	.7	7.2	10.3
Control	9.9	9.9	2.2	3.6	.5	.6	5.0	6.7
			Experiment III					
Control	9.7[1]	9.5[3]	2.8[1]	3.4[3]	.8[1]	.8[3]	5.3[1]	5.1[3]
Fat I[6]	9.9	9.5	3.2	3.8	.7	.7	6.7	6.1
Control	9.5	9.3	2.8	3.5	.6	.7	4.8	4.4
Fat II[5]	9.4	9.2	2.8	3.3	.7	.8	5.2	4.9

[1]Data for KB.

[2]Data for WH.

[3]Data for RH.

[4]Fat from dairy cream.

[5]Fat from corn oil and avocado.

[6]Fat from butter and meat.

during the high fat diets, and the urinary pH was definitely lower (Tables 19 and 24, pages 141 and 148, respectively). Acidosis has been reported to increase calcium loss in the urine (Farquarson et al., 1931; and Lemann et al., 1967), and a high protein diet reportedly gives a negative calcium balance (Anand and Linkswiler, 1974). Furthermore, it has been shown that Eskimos have a relative bone deficiency in calcium (Mazess and Mather, 1973). So apparently, in Experiment II, the increased bone mobilization due to acidosis raised the blood level slightly, but during Experiment III, the phosphorus in the meat appeared to be the dominating factor.

In Experiment II, bilirubin was definitely increased when KB consumed the high fat diets, but there appeared to be no difference in Experiment III. This probably indicates a decreased hemoglobin degradation during the high carbohydrate diets in Experiment II; but despite occasional lysed RBC, during the high fat diets in Experiment III, there did not appear to be an increase in bilirubin above control.

Uric acid showed a significant increase in Experiment II, both when fat came from dairy cream and corn oil; but during Experiment III, there was little, if any, increase in uric acid when the subjects consumed the Fat II diet based on corn oil. The increase in serum uric acid seen in Experiment III when fat came from meat and butter, was less than the increase seen during the high fat, low protein diets (Experiment II). The level of serum uric acid in WH was very high for both control and high fat diets, indicating that this subject had a problem with purine metabolism. For this reason, WH did not participate in Experiment III.

Increased blood uric acid during starvation was reported by Lennox (1924). Christofori and Duncan (1964) concluded that during fasting or consumption of high fat diets, decreased levels of glucose and/or AA in the glomerular filtrate increased uric acid reabsorption in the renal tubules because of decreased competition from these compounds. If this indeed is the case, it can explain the results seen in this study.

D. Metabolic Profiling of Organic Acids in the Urine

When interpreting the excretion of organic acids in the urine, it is important to consider the different sources for an altered excretory pattern. When the diet is altered, certain compounds present or not present in a particular diet, will affect metabolites present in the urine. Furthermore, drastic alterations in the diet are likely to affect the intestinal microflora as reported by Reddy et al. (1975), which can greatly alter the metabolites available for urinary excretion. In addition, the composition of the food consumed can greatly alter the metabolic setting, so that products such as ketoacids are produced in altered amounts; and the secretion of hormones (catecolamines) causes an alteration in the excretion of hormonal degradation products. Finally, alteration can be expected due to individual variation in the metabolic pathways and physiological responses.

From Table 20 (page 143), a basic difference is easily seen between KB and WH when they consumed the same foods in the same amounts. KB had a relatively low lactic acid excretion, but high β-hydroxybutyric acid excretion when consuming the control diets; whereas, the opposite

was true for WH. This indicates that WH had a higher reliance on carbohydrate metabolism than KB, which can also be seen in Figure 1 (page 107) where the RQ's for WH were higher during exercise. There was no difference in β-hydroxybutyrate production during the high fat diets. Training is known to increase reliance on fat metabolism, and on the basis of the RQ values, KB appears to have been in better physical condition. WH, being 6'3" tall and weighing 230 lbs compared to KB's 5'7" and 140 lbs., is about 50 lbs overweight; and it is possible that high carbohydrate substrate utilization is caused by obesity. To my knowledge, such a relationship has not been investigated.

Glycerol excretion was very similar in both subjects, and increased about six-fold during the high fat periods. This should be expected, since fat was the principal substrate during these periods. Phosphate excretion showed no change in KB and even decreased in WH during the high fat dietary periods, despite the elevated plasma levels during that regimen. This seems to indicate an altered renal clearance. Glutaric acid, one of the end products of lysine metabolism, showed no change in KB, but decreased in WH when he consumed high fat diets, probably indicating an alteration in the intestinal microflora (White et al., 1973c). Adipic acid increased tremendously during the Fat I diet (dairy cream) in Experiment II, which probably was due to ω-oxidation of the short chain fatty acids found in butterfat (White et al., 1973b).

Citric acid showed no variation in either subjects, which is interesting, since it has been implicated in acid-base balance (Tischler

et al., 1970). Vanillylmandelic acid (VMA) in the urine was much higher during the control period for both subjects. Being an end product of catecolamine degradation, it could indicate a decreased excretion of catecolamines during the high fat dietary periods; however, the elevated excretion when the control diets were fed, may have resulted from the fact that the bananas in that diet increased the excretion of VMA (Zilva and Pannall, 1975). Variation in hexuronic acid is apparently due to variation in bacterial action and dietary constituents (White et al., 1973). The high excretion of hippuric acid during the CHO periods was probably due to the high content of benzoic acid in the control diet (White et al., 1973).

E. Cholesterol, Triglycerides and HMG

During most high fat periods, the serum cholesterol level increased and then decreased again during the following control period, as seen from Tables 22 and 25 (pages 146 and 151, respectively). The serum cholesterol from the last day of the period probably best represents the effect of the particular dietary regimen. For this reason, the serum cholesterol level (mg/dl) from the last analysis is given in Table 30. Also included is the relative level of hydroxymethylglutarate (HMC) in the urine (the average value for the last two days) from Experiment II, and the serum triglyceride concentration (mg/dl) for the last day of each dietary period in Experiment III.

As expected, cholesterol increased when the fat came from saturated fat; but it was surprising that the serum cholesterol level also increased when the fat came from corn oil, despite the absence of any

TABLE 30. Serum cholesterol, urinary hydroxymethylglutarate, and serum triglycerides.

Date	Diet	Experiment II Subject	Chol[1]	HMG[2]
1/23	CHO I[4]	KB	181	1.97
1/30	Fat I[5]	KB	230	.38
2/ 6	CHO II[6]	KB	184	4.00
2/13	Fat II[6]	KB	225	.25
2/20	CHO III	KB	195	1.05
1/23	CHO I[4]	WH	205	.32
1/30	Fat I[5]	WH	210	.08
2/ 6	CHO II[6]	WH	194	.47
2/13	Fat II[6]	WH	204	.10
2/20	CHO III	WH	220	.22

Date	Diet	Experiment III Subject	Chol[1]	TG[3]
4/16	CHO I[4]	KB	212	
4/29	Fat I[7]	KB	292	82.6
5/14	CHO II[6]	KB	234	73.2
5/28	Fat II[6]	KB	186	98.2
4/16	CHO I[4]	RH	156	
4/29	Fat I[7]	RH	214	76.3
5/14	CHO II[6]	RH	159	83.5
5/28	Fat II[6]	RH	144	73.0

[1]Serum cholesterol (mg/dl) from the last day of the dietary period.

[2]Hydroxymethylglutarate (relative to tropic acid) in the urine.

[3]Triglycerides (mg/dl).

[4]High carbohydrate control diet.

[5]Fat from dairy cream.

[6]Fat from corn oil and avocadoes.

[7]Fat from butter and meat.

cholesterol in the diet. However, this was seen only in Experiment II. Anderson et al. (1971), found no effect of dietary protein on serum cholesterol, provided that the minimum requirement was met. Intakes below the minimum requirement usually reduce plasma cholesterol, as seen in kwashiorkor and semi-starvation (Keys et al., 1950). In Experiment III, a decrease in plasma cholesterol occurred when the subjects were fed the corn oil soy-protein diet. Estimation of cholesterol by the methods routinely used in clinical laboratories is usually not very reliable (White head et al., 1973b); however, the Hycel method used in these experiments has a standard deviation of 2.3 over a 10-day period, and our results appear very consistent.

The serum cholesterol levels in Experiment II seem to be negatively correlated with the urinary excretion (and therefore, presumably the blood level) of hydroxymethylglutaric acid (HMG) (Table 30). It is generally believed that HMGA-CoA reductase is the controlling enzyme in cholesterol synthesis; and this enzyme is competitively inhibited by HMG (Beg and Lupien, 1972). HMG-CoA is converted to HMG by the enzyme hydrolase in an essentially irreversible reaction. It appears that diet affects the level of HMG, which in turn seems to control cholesterol synthesis. This hypothesis needs further investigation. The serum cholesterol and relative urinary HMG concentration for KB during Experiment II are plotted in Figure 5; serum cholesterol and triglycerides during Experiment III are plotted in Figure 6.

The linear regression lines and correlation coefficients for cholesterol and HMG, were calculated for KB and WH during Experiment II.

FIGURE 5. Serum cholesterol and hydroxymethylglutaric acid (HMG) for KB during Experiment II. (The control diet was a high carbohydrate diet. During Fat I the fat came primarily from dairy cream and during Fat II, primarily from corn oil and avocadoes. The concentration of HMG is relative to the internal standard.)

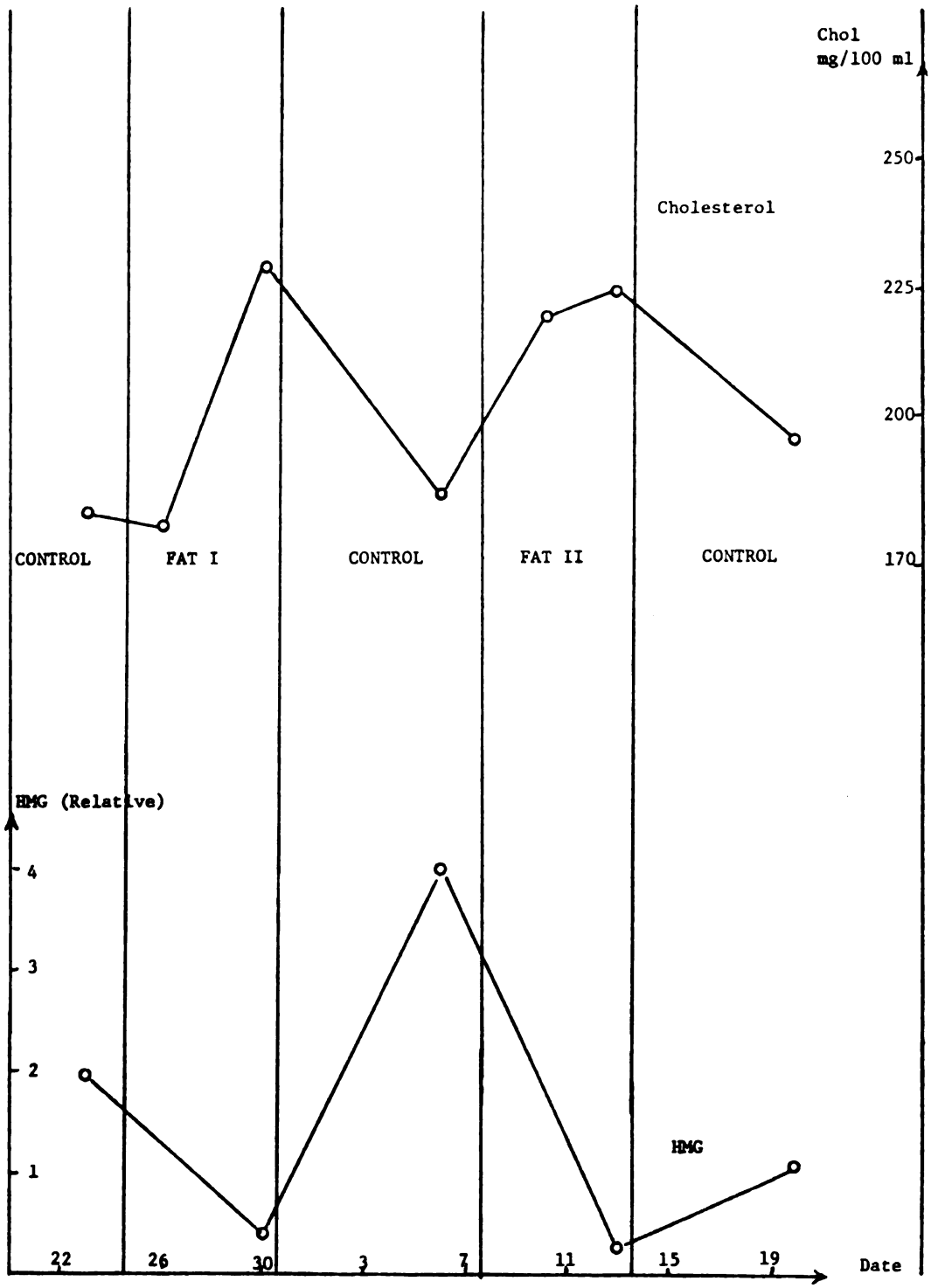
Chol
mg/100 ml
250
225
200
170
Cholesterol
CONTROL
FAT I
CONTROL
FAT II
CONTROL
HMG (Relative)
4
3
2
1
HMG
22
26
30
3
7
11
15
19
Date

FIGURE 5

FIGURE 6. Plasma cholesterol and triglycerides for KB in Experiment III. (CHO indicates the high carbohydrate control diet. During Fat I the fat came primarily from dairy cream and during Fat II, primarily from corn oil and avocadoes.)

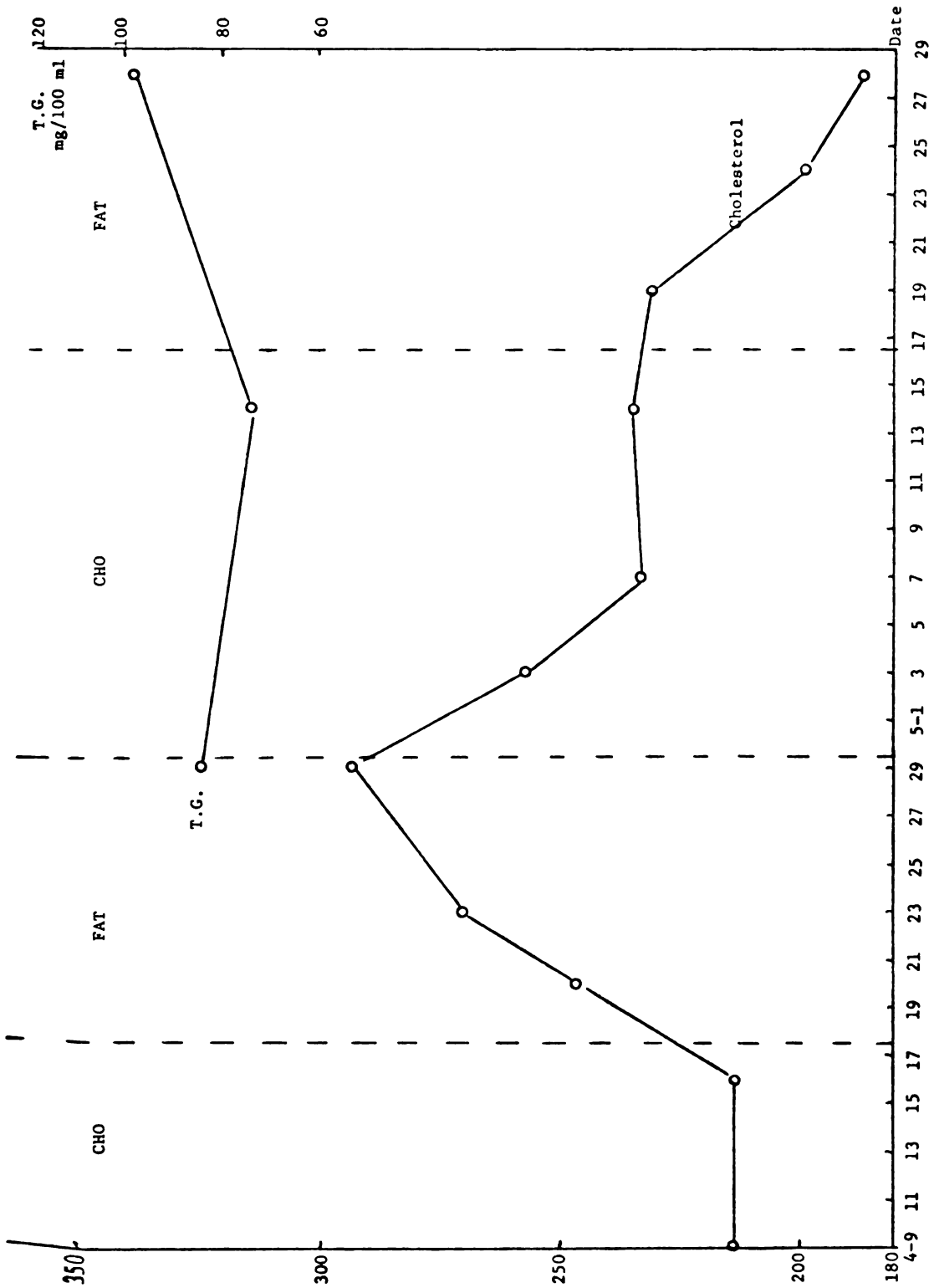
T.G.
mg/100 ml
120
100
80
60
FAT
CHO
FAT
CHO
T.G.
Cholesterol
Date
350
300
250
200
180
4-9
11
13
15
17
19
21
23
25
27
29
5-1
3
5
7
9
11
13
15
17
19
21
23
25
27
29

FIGURE 6

For KB:

$$Chol = -11.8\ HMG + 221.1;\ r = -0.79$$

For WH:

$$Chol = -33.1\ HMG + 214.5;\ r = -0.57.$$

The poor correlation for WH can be expected, since he showed relatively little variation in either variable as the diet was altered.

The serum triglyceride levels during the high protein study were not related to the cholesterol level; and for KB they decreased when the control diet was consumed, but for RH, they increased. Glueck et al. (1969) found that the average rise in TG was 100 mg/ml in normal people when they changed from a diet containing 40% of the calories from carbohydrate to one with 80% of the calories from carbohydrates. However, people who subsist on starchy foods do not appear to have elevated TG levels (Florey et al., 1973). Antonis and Bersohn (1961) found that when white Europeans changed from a European to an African diet (based on corn), the plasma TG rose and cholesterol fell; however, after eight months on the African diet, the TG-levels had returned to normal. Since KB habitually consumes a high carbohydrate diet, no increase in plasma TG would be expected. In RH there was not much variation either, but at least the change was in the anticipated direction. It should be pointed out that all results are well within the normal range.

F. Body Weight Loss and Water-Balance

Using the data in Table 24 (page 148), the average water-balance (WB) from the three control (CHO) periods and from each of the high fat

periods was calculated. The results for Experiment II are given in Table 31, and in Table 32 for Experiment III.

TABLE 31. Water-balance and body weight loss during Experiment II.

	Data for KB		Data for WH	
Diet	Water-Balance[1]	Weight Loss[2]	Water-Balance[1]	Weight Loss[2]
Control[3]	1283	0.0	2142	0.0
Fat I	478	3.2	1226	5.5
Fat II	520	2.0	925	5.5

[1]Relative water-balance in g/24 hrs.
[2]Weight loss in kg for the entire dietary period.
[3]Mean for the three CHO periods.

TABLE 32. Water-balance and body weight loss during Experiment III.

	Data for KB		Data for RH	
Diet	Water-Balance[1]	Weight Loss[2]	Water-Balance[1]	Weight Loss[2]
Control[3]	1435	0.0	1457	0.0
Fat I	603	3.0	489	2.0
Fat II	826	1.8	1423	1.6

[1]Relative water-balance in g/24 hrs.
[2]Weight loss in kg during the entire dietary period.
[3]Mean for the two CHO periods.

In Table 33 the actual weight loss (ΔW_1) is compared with the weight loss based on the water-balance (ΔW_2), and the maximal loss

TABLE 33. Total weight loss during each dietary period.

Study	Diet	ΔW_1[1]	ΔW_2[2]	ΔW_3[3]	Subject
Experiment II[4]	Fat I	3.2	4.0	3.0	KB
	Fat II	2.0	3.8	3.1	KB
	Fat I	5.4	4.6	4.2	WH
	Fat II	5.4	6.1	4.4	WH
Experiment III[5]	Fat I	3.0	9.2	3.5	KB
	Fat II	1.8	6.7	4.5	KB
	Fat I	2.0	10.6	3.9	RH
	Fat II	1.6	0.3	4.1	RH

[1]The observed weight loss in kg for the entire period.

[2]The weight loss calculated from the water-balance data.

[3]Weight loss calculated on the basis of estimated glycogen degradation and calculated lean tissue catabolism (see text for explanation).

[4]Each period lasted five days.

[5]Each period lasted eleven days.

calculated from the estimated (max.) glycogen loss and the lean tissue loss (ΔW_3). ΔW_2 was calculated from the average water-balance during the control period minus the average water-balance during each of the fat periods multiplied by the number of days the subjects were on the particular diet. ΔW_3 was calculated from the loss due to glycogen depletion, as found by Olsson and Saltin (1970) and the lean tissue loss due to protein catabolism estimated from the difference between the mean nitrogen excretion during the fat periods and the mean nitrogen excretion during the control periods. The value was converted to protein, multiplying by 6.25, and to lean tissue mass, multiplying the latter by 5. This figure was then multiplied by the number of days the subject consumed the diet.

From Table 33 it is seen that the weight loss calculated from the water-balance results is not very reliable. The two primary reasons for this are fluctuation in the actual water loss (sweat and insensible perspiration), and variation in stool moisture content. From Table 27 (page 154), it is seen that the actual water loss varied from about 1100 (ignoring one very low value of about 700) to about 1700. Even if the actual water loss was unaffected by the diet, but the mean water loss is in error by 300 ml/day, it would give an accumulated error of more than 3 kg for an 11-day period. The stool volumes on the high carbohydrate diets were usually 500 g or more, compared to almost no stools during the saturated fat diet (Fat I) though some subjects had occasional diarrhea); and a small but consistent stool during the unsaturated fat diet (Fat II). Assuming 80% water in the stool, this could account for

a difference of about 400 g water/day, or about 4.5 g for 11 days. Taking these variations into consideration, the inconsistency between ΔW_1 and ΔW_2 can be explained.

Water intake and urine output are plotted in Figure 7 and in Figure 9 for KB during Experiments II and III, respectively; and in Figure 11 for RH during Experiment III. Generally, the difference became much less during the high fat diet, indicating that endogenous water was available. Figures 8, 10, and 12 show the total water-balance, and the body weight. In Experiment II, the water-balance reached the lowest value at the beginning of the study, and then gradually increased toward the control value; but during Experiment III, there is no consistent change throughout the study.

This indicates first of all, that there was no correlation between the degree of water loss and urinary excretion of ketones, since ketone excretion was at control level at the beginning of the period, but increased toward the end. The large water loss on the first day was probably due in part to liberation of the water associated with liver glycogen (about 350 ml), which is depleted within the first 24 hours (Hultman et al., 1974; and Bergström and Hultman, 1972). In Experiment III, the water-balance was followed during the transition from the high fat to high carbohydrate diet, and as seen from Figures 10 and 12 and Table 27 (page 154), there was an increased water retention the first few days, indicating resynthesis of glycogen.

Secondly, the more constant water-balance during the high fat periods in Experiment III may indicate a glycogen sparing effect of the

FIGURE 7. Water-balance for KB during Experiment II. (CHO indicates the high carbohydrate control period. During Fat I the fat came primarily from dairy cream and during Fat II, primarily from corn oil and avocado. Total water intake includes both drinking water and water contained in the food. Urine is the 24-hour urine volume.)

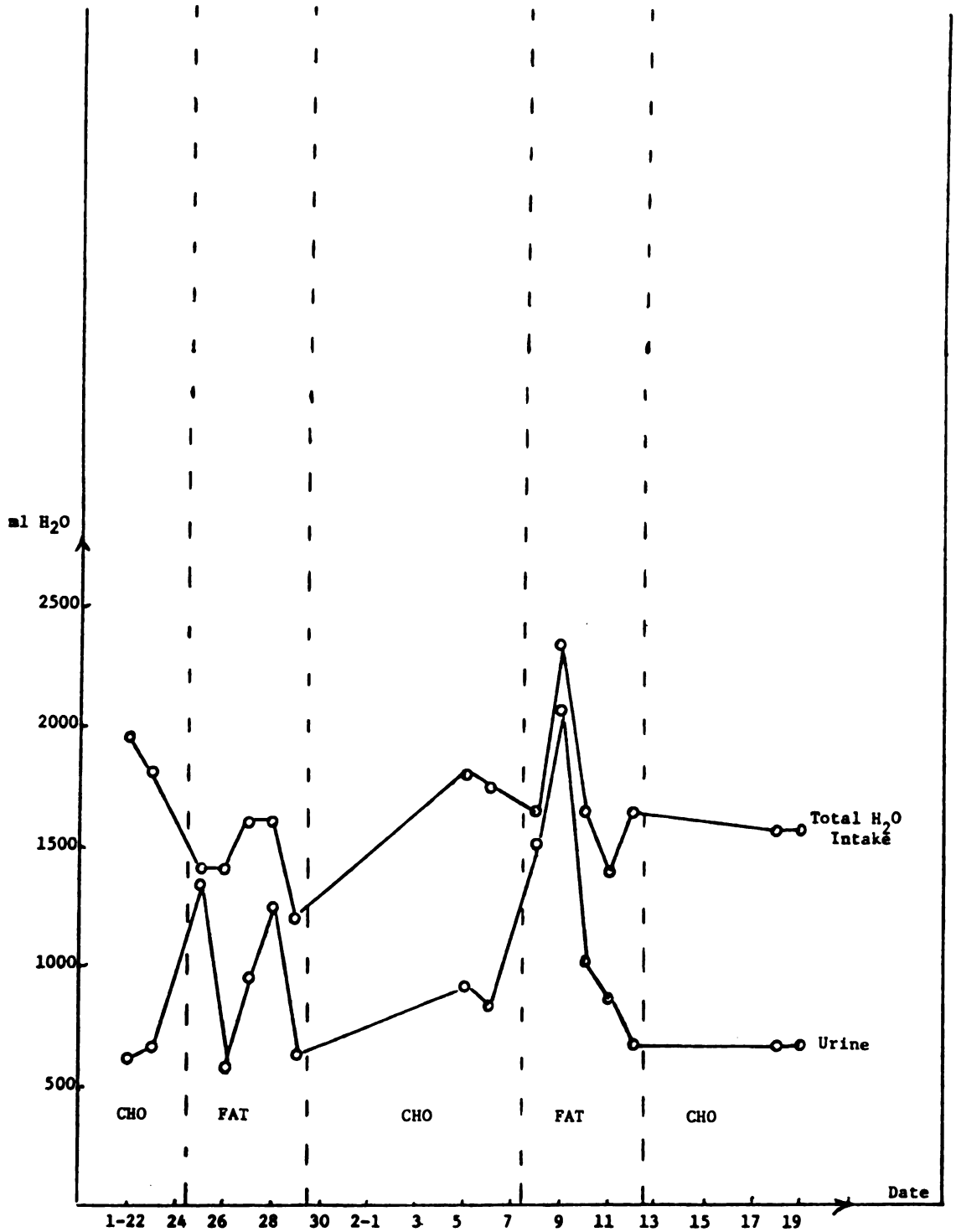
ml H_2O
2500
2000
1500
1000
500
Total H_2O Intake
Urine
CHO
FAT
CHO
FAT
CHO
Date
1-22
24
26
28
30
2-1
3
5
7
9
11
13
15
17
19

FIGURE 7

FIGURE 8. Body weight, ketone excretion, and water-balance for KB during Experiment II. (CHO indicates the high carbohydrate control periods, and Fat, the high fat dietary periods. Body weight is the cumulative weight. Ketones indicates serum concentration above control values (determined by the nitroprusside reaction). Water-balance is the difference between the total water intake and the 24-hour urine volume.)

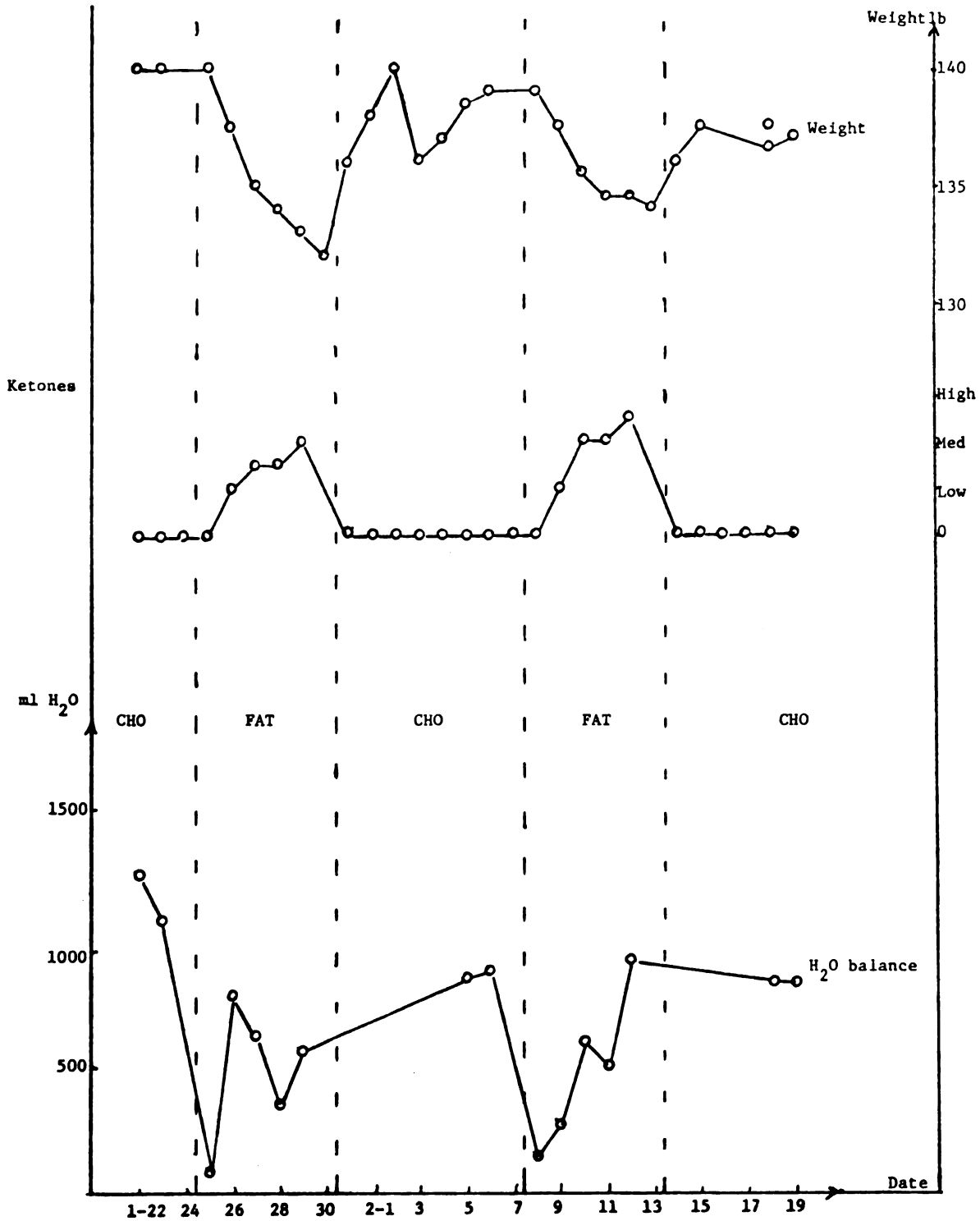
Weight lb
140
135
130
Weight
Ketones
High
Med
Low
0
ml H_2O
CHO
FAT
CHO
FAT
CHO
1500
1000
500
H_2O balance
Date
1-22
24
26
28
30
2-1
3
5
7
9
11
13
15
17
19

FIGURE 8

FIGURE 9. Water-balance for KB during Experiment III. (CHO indicates the high carbohydrate control period. During Fat I, the fat came primarily from meat and butter and during Fat II, primarily from corn oil and avocado. Total water includes both drinking water and water contained in the food. Urine is the 24-hour urine volume.)

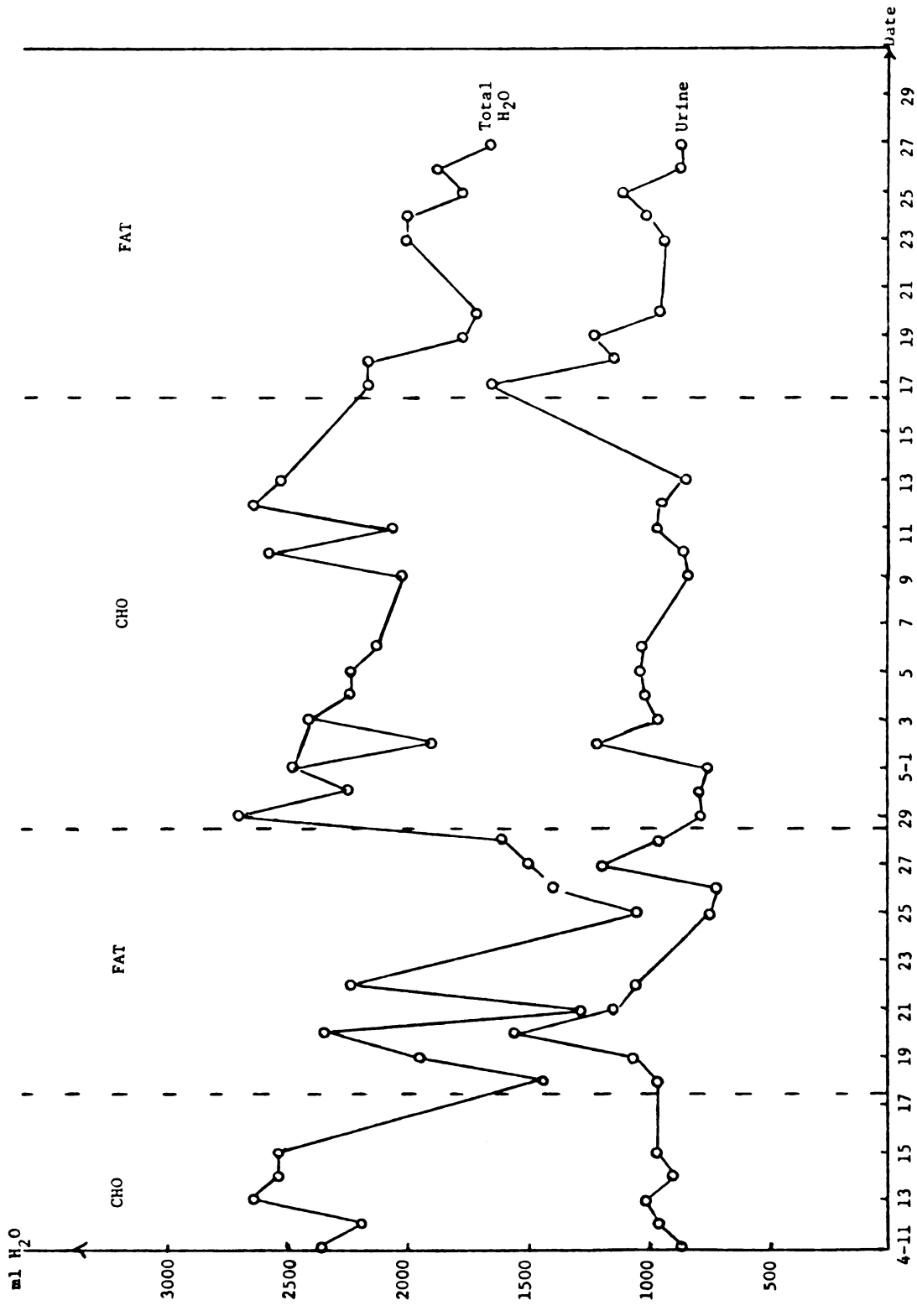
ml H_2O
3000
2500
2000
1500
1000
500
CHO
FAT
CHO
FAT
Total H_2O
Urine
4-11 13 15 17 19 21 23 25 27 29 5-1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Date

FIGURE 9

FIGURE 10. Body weight and water-balance for KB during Experiment III. (CHO indicates the high carbohydrate control period. Fat I was based on butter and meat, and Fat II was based on corn oil and avocado. Body weight is in pounds and water-balance is the difference between the total water intake and the urine volume.)

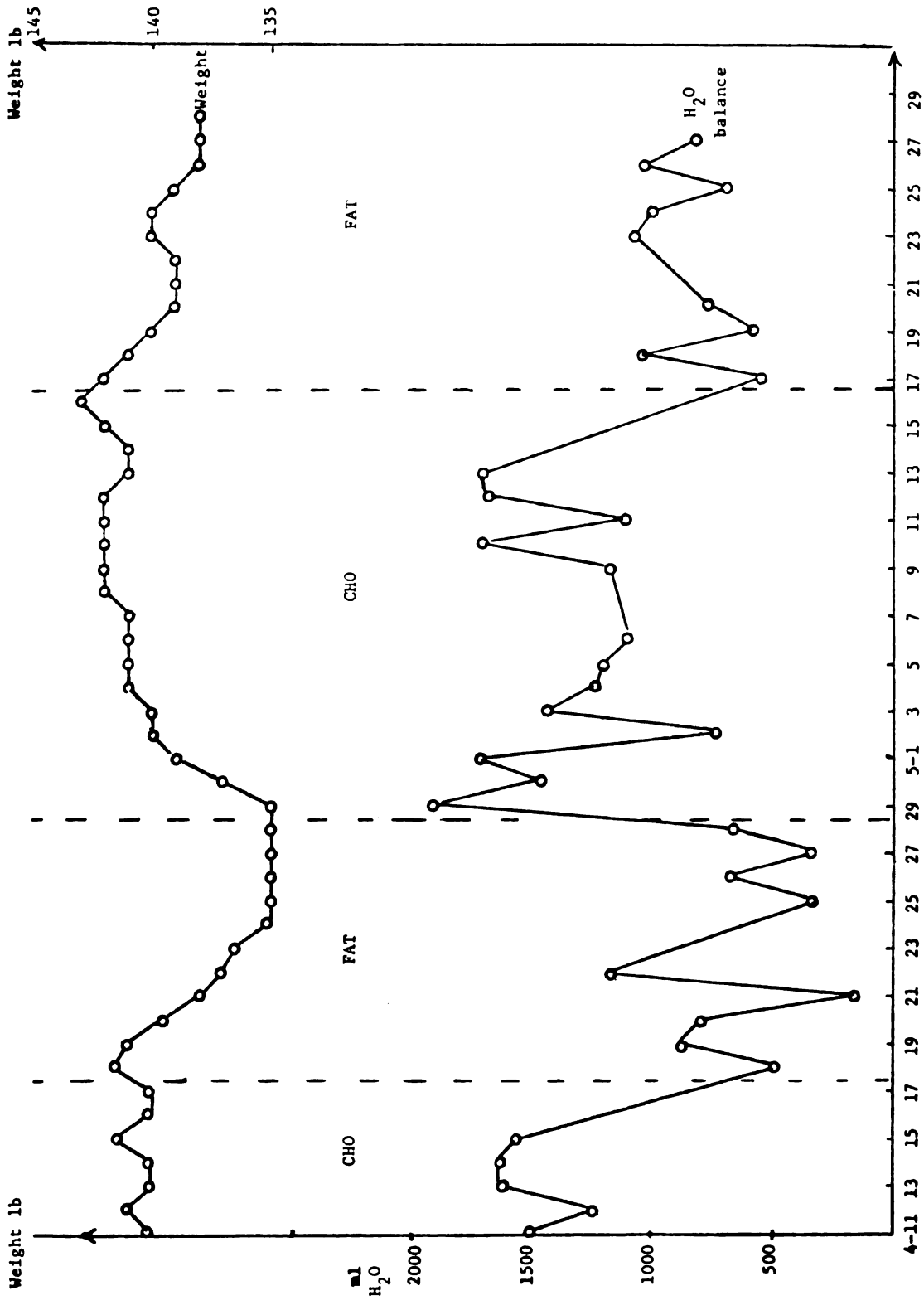
Weight lb
145
140
135
Weight
FAT
CHO
H_2O balance
Weight lb
CHO
FAT
ml H_2O
2000
1500
1000
500
4-11 13 15 17 19 21 23 25 27 29 5-1 3 5 7 9 11 13 15 17 19 21 23 25 27 29

FIGURE 10

FIGURE 11. Water-balance for RH during Experiment III. (CHO indicates the high carbohydrate control period. Fat I was based on butter and meat, while Fat II was based on corn oil and avocado. Total water intake includes both drinking water and water contained in the food. Urine is the 24-hour urine volume.)

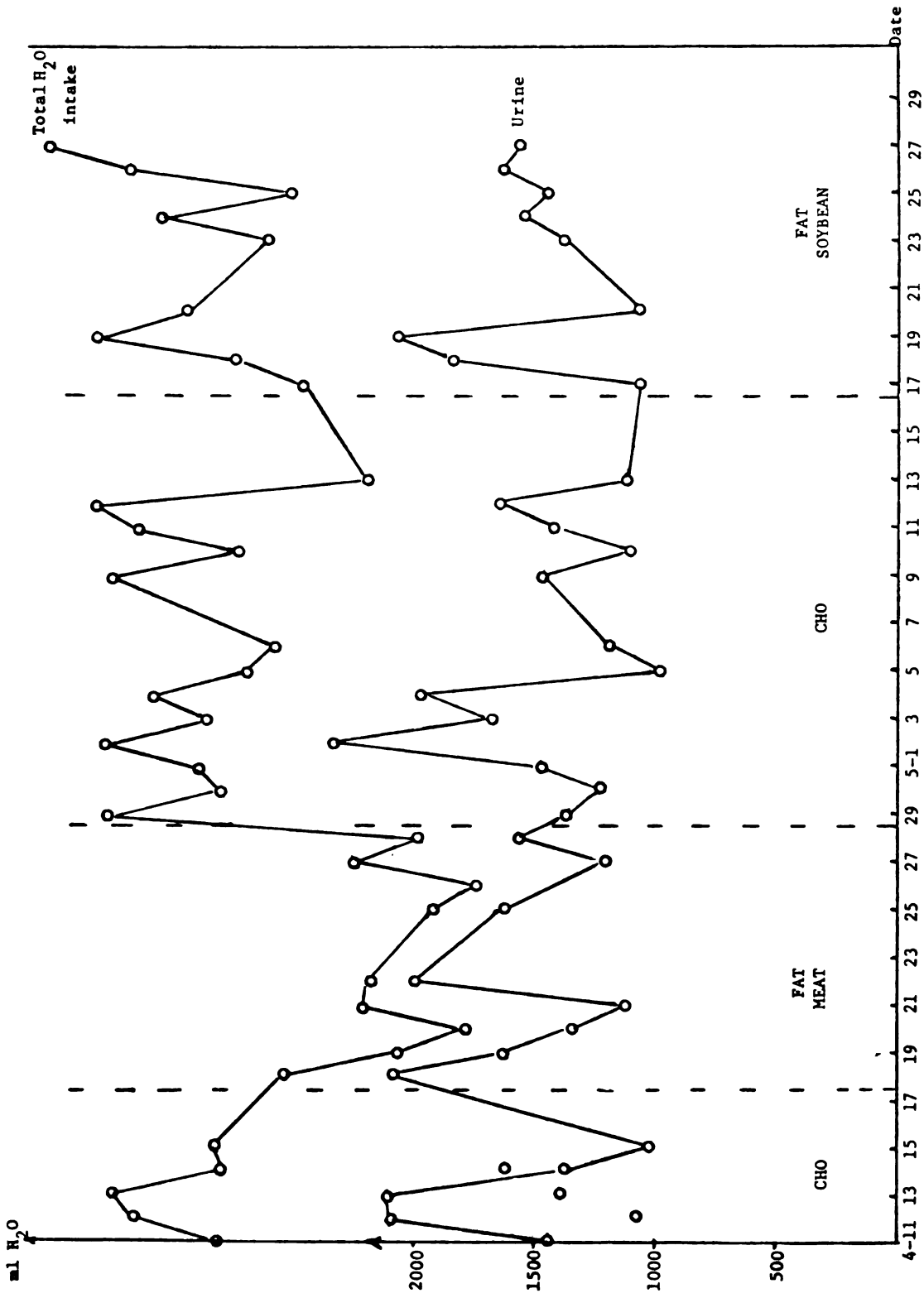
ml H_2O
2000
1500
1000
500
Total H_2O intake
Urine
CHO
FAT MEAT
CHO
FAT SOYBEAN
4-11 13 15 17 19 21 23 25 27 29 5-1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Date

FIGURE 11

FIGURE 12. Body weight and water-balance for RH during Experiment III. (CHO indicates the high carbohydrate control period. Fat I was based on butter and meat; Fat II was based on corn oil and avocado. Body weight is in pounds, and water-balance is the difference between the total water intake and the urine volume.)

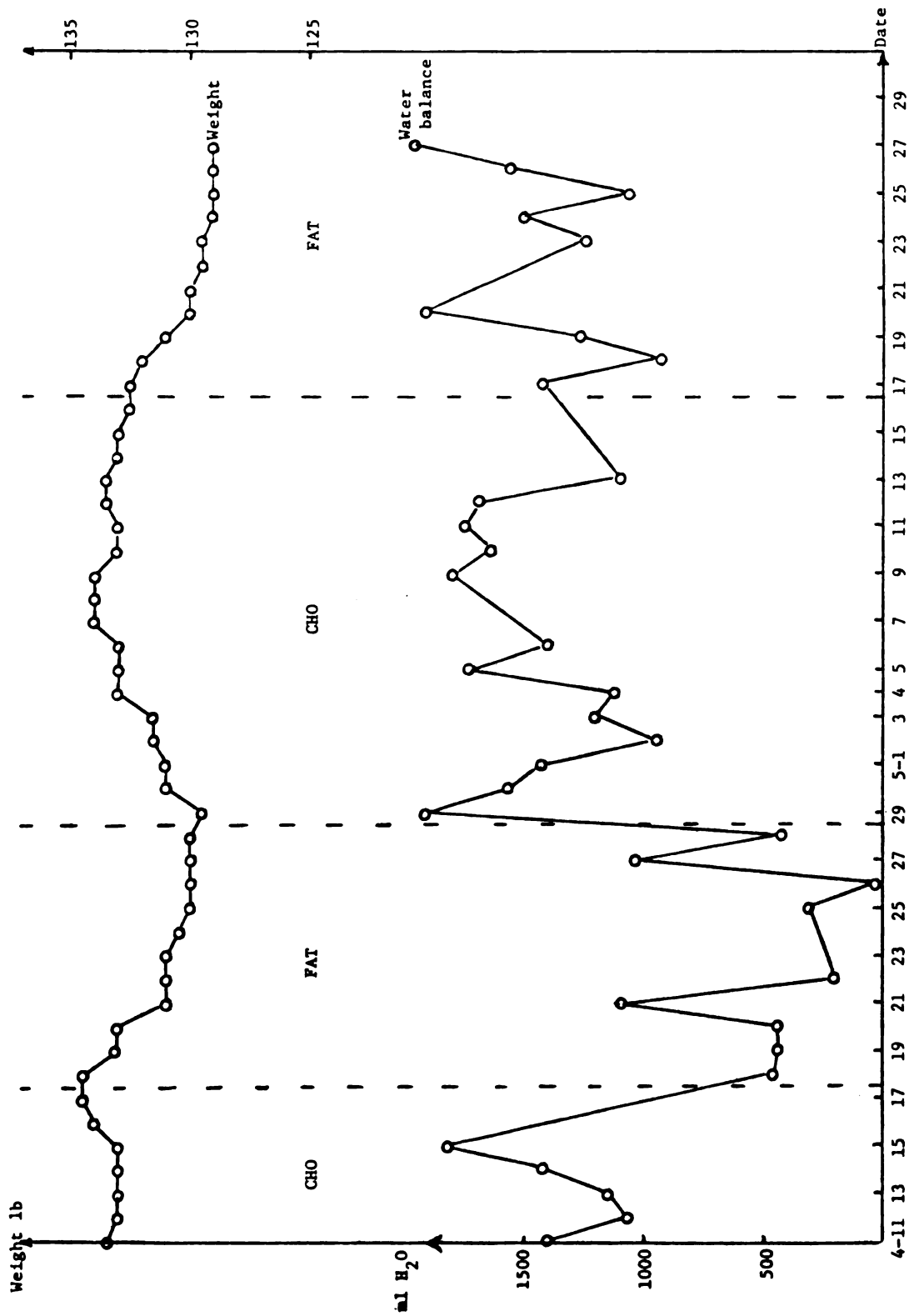
Weight lb
135
130
125
Weight
Water balance
CHO
FAT
CHO
FAT
ml H_2O
1500
1000
500
4-11 13 15 17 19 21 23 25 27 29 5-1 3 4 5 7 9 11 13 15 17 19 21 23 25 27 29
Date

FIGURE 12

protein compared to Experiment II. Additional support for this concept comes from the decreased rate of weight loss during the high fat diets in Experiment III (Table 33, page 182). That the decreased weight loss during the high protein diet (Experiment III) was due largely to a glycogen sparing effect rather than a protein sparing effect, is indicated by a similar increase in urinary nitrogen excretion during the high fat diets in Experiments II and III. The caloric content and physical activities were unaltered during these studies. If high dietary protein levels indeed have a glycogen sparing effect, this could be important for endurance performance.

From Figure 8 (page 188), it is seen that the rate of weight loss was highest in the first part of the study periods, when the water-balance was lowest; however, the rate of weight loss was also highest in the first part of the high fat periods during Experiment III, but in this case, there was no increase in the water-balance. From Table 33 (page 182), it is seen that the weight loss calculated from the water-balance data is grossly in error, probably due to the accumulation of errors during the longer periods of this study.

When the actual weight loss is compared with the weight loss based on glycogen and lean tissue mass, it is seen from Table 33 that in Experiment III, the calculated weight loss (ΔW_3) was always higher than the actual weight loss (ΔW_1), indicating the possibility of a glycogen sparing effect during a high protein diet. Hultman and Nilsson (1975) have shown that in subjects fed a low carbohydrate diet, the liver glycogen is not replenished as it is in some animal species, indicating

a lower gluconeogenic capability in man. In the resting state, muscle glycogen is only slowly depleted during starvation (about 50% in four days) according to Hultman and Nilsson (1975). The rate of muscle depletion may very well depend upon the level of protein intake, since the branched chain amino acids apparently are oxidized for energy utilization in the muscle; and the AA profile appears to control the release of alanine from the muscle (Felig, 1975; and Cahill, 1976).

During Experiment II, it is apparent that the weight loss calculated from glycogen and lean tissue mass catabolism (ΔW_3) was less than the actual weight loss (ΔW_1) in most cases. This could possibly be explained by glycogen super-compensation during the high carbohydrate period (Bergström and Hultman, 1966). However, considering the relatively short dietary periods, it is not very likely that the muscle glycogen was depleted (Hultman and Nilsson, 1975), indicating that part of the weight loss had a different origin.

G. Dehydration

The plasma albumin (Alb) and total protein (TF) concentration on the last day of each experimental period are listed in Table 34. The average value for the three control periods and the average from the two fat diets are used. It is seen that for KB, the plasma albumin increased 9% on the high fat diet, while the total protein increased 11%. For WH, the respective values are 4% and 10%. These results seem to indicate a 10% decrease in the extracellular fluid volume (EFV).

During the Experiment III, more blood samples were taken and the dietary periods were longer; for this reason, the mean albumin (Alb) and

TABLE 34. Plasma albumin and total protein concentration at the end of the control and fat periods during Experiment II.

Diet	Data for KB		Data for WH	
	Alb[1]	TP[2]	Alb[1]	TP[2]
Control[3]	4.4	6.4	4.7	7.1
FAT[4]	4.9	7.1	4.9	7.8

[1]Serum albumin (g/dl).

[2]Total protein in serum (g/dl).

[3]Average for the last day of the three CHO periods.

[4]Average for the last day of the two fat periods.

the mean of the total protein concentration in the blood were calculated for the fat periods and for the control periods (from Table 25, page 151), and the results are shown in Table 35. No systematic change is seen in Table 25, and the data in Table 35 shows no indication of dehydration during the high fat periods of Experiment III.

TABLE 35. Mean plasma albumin and mean total protein concentration for the control and fat periods during Experiment III.

Diet	Data for KB		Data for RH	
	Alb[1]	TP[2]	Alb[1]	TP[2]
Control[3]	4.0	6.4	4.2	6.9
FAT[4]	4.0	6.5	4.3	7.1

[1]Serum albumin (g/dl).

[2]Total proteins in serum (g/dl).

[3]Average for the two CHO periods.

[4]Average for the fat periods.

Sodium, potassium, and chloride concentration (Table 25) were determined in the blood the last day of each dietary period, and there was a tendency for these values to be higher on the saturated fat meat diet; but in view of the few observations, this is probably not important.

The hematocrit (HTC) and hemoglobin data for Experiment III are given in Table 36. No consistent variation between the different periods was seen. Costill et al. (1974a) have shown that the mean corpuscular volume (MCV) decreases because of dehydration (increased osmolality of the blood), and that this shrinkage is accurately described by the mean corpuscular hemoglobin concentration (MCHC). Consequently, dehydration as measured by changes in the HTC will be erroneous unless corrections are made for changes in the MCV. In the present study, no systematic changes were observed in either of these variables, as seen from Table 26 (page 153). The mean values for the fat periods and for the control periods are given in Table 36; and as seen, there is no difference, indicating again, that no dehydration was detected during the high fat period of Experiment III.

TABLE 36. Mean hematocrit data during Experiment III.

	Data for KB			Data for RH		
Diet	HTC[1]	MCV[1]	MCHC[1]	HTC[1]	MCV[1]	MCHC[1]
Control	41	85	29	43	86	34
FAT	41	84	29	43	86	35

[1]For explanations of abbreviations, see page 139.

From changes in plasma proteins, it seems that a dehydration affecting the EPV occurred during Experiment II, whereas none was observed during Experiment III. Unfortunately, no hematocrit data were available for the former period. Gamble et al. (1923) showed that water is lost from the body during fasting, and concluded that it is due to destruction of protoplasm and reduction of tissue glycogen. Bloom and Azar (1963) observed a prompt weight loss associated with a negative sodium balance during starvation and when carbohydrate free diets were fed, regardless of the amount of salt consumed by the subjects. In the present study, it is possible that the sodium intake was limited during Experiment II. These diets were semi-synthetic mixtures to which no sodium was added, nor generally used by the subjects. This is in contrast to the more natural diets used in the Experiment III, where the subjects made liberal use of salt to improve the taste. This probably explains the high sodium level seen in the blood during Experiment III (Table 25, page 151). However, if we accept the data of Bloom and Azar (1963), we can exclude differences in sodium intakes as a cause for the possible dehydration during the fat periods of Experiment II. This is in contrast to the results of Maagøe (1968); and Veverbrants and Arky (1969).

Bloom et al. (1966) found that carbohydrate deficiency during total fasting caused a decrease in extracellular fluid volume (including blood volume), as determined from serum protein and hematocrit. In contrast to the results of Bloom and Azar (1963), the weight loss in the present study appeared to depend on the protein intake; but the

diets employed by Bloom and Azar were carbohydrate free, which could make a difference. Katz et al. (1968) found an increased salt excretion during starvation, which was not abolished by giving supplementary salt comparable to that contained in a normal diet. When the salt intake was kept constant, either carbohydrate or protein plus fat prevented the salt loss; however, carbohydrate feeding also abolished the ketosis, whereas protein feeding did not. These results support our finding that the amount of proteins in the high fat diets determined whether or not they caused dehydration; and that dehydration is not caused by ketosis.

One possible explanation for these results is that carbohydrate deprivation causes depletion of the glycogen stores in the kidney, so that the supply of glucose is insufficient to operate the sodium-potassium pump at a sufficient rate to prevent sodium loss. Hoffman (1973) has shown that there is a special compartment of ATP that is preferentially used by one component of the ATP'ase. In the red blood cell, this ATP is derived from anaerobic glycolysis (Parker and Hoffman, 1967). The saline diuresis appears to be related to the decrease in the insulin/glucagon ratio (DeFronzo et al., 1975; and Spark et al., 1975). It may be that the low insulin/glucagon ratio prevents sufficient glucose from entering the renal cells, and this may limit Na reabsorption. As carbohydrate deprivation proceeds, kidney gluconeogenesis may supply glucose internally, and this gluconeogenesis may be accelerated by a high protein intake. A similar explanation has been suggested by Wright et al. (1963).

Cizek et al. (1977), from experiments with rabbits, suggest that the sodium loss is due to decreased bicarbonate in the blood; but that is difficult to understand because the mechanism commonly used to explain urine acidification is through exchange of sodium with hydrogen ions. These hydrogen ions are then buffered by bicarbonate ions or HPO_4^{--}, with the effect of excreting $H_2PO_4^-$ and reabsorbing HCO_3^- (as CO_2). If these buffers, in very severe acidosis, become depleted, ammonia is used as a buffer (Gottschalk and Lassiter, 1974). Cizek et al. (1977) studied the effect of water deprivation on the sodium loss during starvation and found it to be independent of water intake; but if rabbits function in a similar manner, an increased urine volume would be expected, regardless of water intake (endogenous water). There is no reason to expect that water deprivation would abolish the loss of sodium.

No sign of dehydration was found (by the methods employed) during Experiment III. Weight loss of the subjects is adequately explained by catabolism of glycogen and lean tissue mass. This was shown by Garrow (1974), who recalculated the results of many studies in the literature in view of the present knowledge about body glycogen stores and their associated water. He concluded that the weight loss during weight reduction could be adequately explained on the basis of glycogen depletion lean tissue, and fat tissue catabolism. During Experiment II, there was some evidence of a decrease in the extracellular body fluid (about 10%). Assuming 15 kg of water, this could mean a weight loss of 1.5 kg for KB. Looking at the weight losses in Table 33 (page 182), it would mean that in order to explain the total weight loss (ΔW_1), one need only assume

that about one-half of the glycogen stores were depleted, which probably could be expected during the five days of carbohydrate starvation.

IX. SUMMARY

In conclusion, it may be said that there was some evidence of dehydration during the low protein, high fat periods (Experiment II), but not during Experiment III, at least not of a magnitude that would have any influence on physical performance. The dehydration in Experiment II was presumably related to sodium excretion caused by carbohydrate deprivation; the exact mechanism of this is unknown but it seems to depend upon the protein intake. With adequate protein intake, there was no evidence of dehydration, and the weight losses observed are adequately explained by glycogen and lean tissue catabolism. The ketosis seen during the high fat periods was not severe, was independent of the nature of fat and protein, and only slightly dependent upon the amount of protein eaten. The level of ketosis was comparable to that observed in most studies of this nature. The serum cholesterol level during the low protein study (Experiment II) did not appear to be affected by the nature of the fat ingested, but was found to be correlated to the amount of hydroxy-methylglutaric acid excreted in the urine.

SUGGESTIONS FOR FURTHER RESEARCH

It is clear from the literature that starvation causes a water loss not easily accounted for. As has been confirmed by the present studies, a somewhat similar loss was observed in subjects consuming a low carbohydrate diet. The negative water-balance is caused by carbohydrate deprivation, but the exact mechanism whereby this occurs is not apparent. The present studies indicate that dehydration is related to the level of protein intake (in addition to the carbohydrate content of the diet), since dehydration was observed only when the intake of both carbohydrate and protein was very low.

Many explanations for the body weight loss seen during a low carbohydrate regimen are given. Some of the more plausible are: Catabolism of glycogen and lean body tissue, dehydration, ketosis, and sodium loss. Because it is impossible to introduce a carbohydrate free dietary regimen without causing a decrease in glycogen stores and lean tissue mass, it is difficult to determine the factor(s) that is (are) responsible for the weight loss.

It appears that dehydration can be caused by either salt restriction (starvation), or a diet low in carbohydrate and protein. To separate these two possible factors, the following experiment is proposed: Subjects should be fed a normal control diet with a

sufficiently high but pre-determined salt intake. When their metabolism has stabilized, a high fat dietary regimen should be introduced. Both carbohydrate and protein intake must be kept low (5% of calories or less), with the same amount of salt as during the control period. The salt balance should be determined, and changes in the various water compartments monitored with tracer dilution techniques. When the study period is completed, it should be repeated without any (or very low) salt intakes.

Similar experiments can be performed with higher levels of carbohydrate and protein (with and without salt restriction), raising first one and then the other to 15 or 20% of calories. With this experimental design, it should be possible to distinguish the effect of a salt restricted diet from that of a diet low in carbohydrate and protein.

The effect of glycogen and lean tissue catabolism on the body weight loss can be studied in the following way: Body weight and nitrogen balance are determined for subjects fed a normal control diet. At the end of this period, muscle- and liver-biopsies can be taken to determine the glycogen content, and the total muscle mass estimated by total body potassium determinations. Total body water can be determined with tritium labelled water, and the plasma volume with I^{131} marked albumin.

The glycogen stores should then be depleted (as far as possible) by having the subjects exercise to exhaustion during an endurance type performance, such as running on a treadmill at about 75% of the maximal oxygen consumption. Determination of the water spaces, muscle and liver

glycogen, and body weight measurements should be recalculated. After the subjects have been allowed to stabilize for about three days while consuming a low carbohydrate, high protein diet, a nitrogen balance should be carried out and muscle- and liver-glycogen, body weight measurement and body water determinations made as before.

Finally, the subjects are fed a high carbohydrate diet for four days and all measurements repeated. The protein intake should be kept constant and relatively high throughout all the dietary periods, and nitrogen balance and body weight measurements carried out daily.

From this experiment (with about ten, physically uniform subjects), the contribution of glycogen stores, lean tissue, and water to the weight loss observed during a high fat, low carbohydrate dietary regimen, could be determined. If the biopsy technique is not feasible in man, a suitable animal model might be selected.

The relationship between substrate (fat or carbohydrate) and the muscular work efficiency can best be studied in an experiment with short work periods (10-20 minutes) and low work intensities (about 30% of the maximal oxygen consumption). The present experiments have shown that under such conditions, the substrate utilization is easily influenced by the dietary regimen; and the glycogen stores are not depleted. It therefore appears possible to separate the effect of using fat as substrate for muscle energy, from other effects of feeding a high fat diet, such as ketosis, dehydration, and glycogen depletion. This type of experiment should be done in the post-absorptive state to eliminate any possible effects of the subjects being in the absorptive state.

It is preferable to have subjects of uniform age, weight, and particularly, physical condition.

During Experiment II (low protein), a high content of polyunsaturated fats in the diet appeared to increase the serum cholesterol level. Since this increase was not seen during Experiment III (high protein), it could not have been caused by the high fat intake, but the increased muscle catabolism during the high fat diet could possibly be a factor. However, a fairly good negative correlation ($r = -0.79$) was found with the urinary excretion of hydroxy-methylglutaric acid. This possibility needs further study.

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APPENDICES

APPENDIX A

Composition of the high carbohydrate control diet for Experiment I (approx. 10% of calories from protein and 75% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein	CHO[2]	Fat[2]
Soyamel[4]	50	247	2	9	28	12
Strawberries	100	37	90	1	10	1
Banana	100	85	76	1	22	0
Orange	100	49	86	1	13	0
Lemon	50	13	45	0	5	0
Grapefruit	150	66	132	1	18	0
Granola[5]	50	230	3	6	34	9
Breakfast Total	600	727	436	19	130	22
Mixed Vegetables[6]	481	149	438	5	37	0
Baked Potatoes[6]	150	140	113	4	33	0
Sweet Potatoes[6]	241	274	171	2	67	0
Fruit Cocktail[6]	411	200	329	2	78	0
Soyameat[7]	100	232	110	18	11	13
Dinner Total	1383	1095	1161	31	226	13
Vegetable Soup[6]	305	201	259	4	37	6
Apple	100	60	84	0	16	1
Bread	107	236	46	12	47	2
Cake	50	150	18	2	26	4
Honey	50	165	8	0	41	0
Jam	50	16	16	0	53	0
Supper Total	662	815	431	18	200	13
TOTAL	2645	2637	2028	68	556	48

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast. The same diet was used for all CHO periods.

[2]Weight in grams.

[3]Calories in kcal.

[4]Fortified Soyamel, Worthington Foods, Worthington, Ohio.

[5]Kellogg Company, Battle Creek, Michigan.

[6]Food Club, Meijer, Inc., Grand Rapids, Michigan.

[7]Soyameat, Salisbury Steak style, Worthington Foods.

APPENDIX B

Composition of the Fat I diet during Experiment I.[1] (approx. 10% of calories from protein and 15% from carbohydrates.)

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Granola[4]	25	124	0	3	16	5
Soya Protein[5]	20	76	0	19	0	0
Orange	50	25	43	0	6	0
Fresh Fruit	100	40	90	0	5	0
Corn Oil	60	540	0	0	0	60
Avocado[9]	100	171	74	2	4	17
Breakfast Total	355	976	207	24	31	82
Baked Potatoes	50	47	38	1	10	0
Soyameat[6]	100	212	62	17	10	11
Dressing[7]	150	736	56	3	8	83
Fruit Cocktail[8]	50	38	18	0	10	0
Whipped Topping	25	65	15	0	5	5
Dinner Total	375	1098	189	21	43	99
Bread	54	118	23	6	24	1
Peanut Butter	25	145	0	7	5	13
Cream Cheese	100	353	54	7	4	35
Dressing[7]	50	246	19	1	3	28
Supper Total	229	862	96	21	36	77
TOTAL	959	2911	497	66	108	256

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of greakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Kellogg Company, Battle Creek, Michigan.

[5]Soya Protein, Fearn Soya Foods, Melrose Park, Illinois.

[6]Soyameat, Wham style, Worthington Foods, Worthington, Ohio.

[7]For composition of the dressing, see Appendix D.

[8]Food Club, Meijer, Inc., Grand Rapids, Michigan.

[9]Fuerte variety (Calavo), California.

APPENDIX C

Composition of the Fat II diet during Experiment I (approx. 6% of calories from protein and 9% from carbohydrates).[1]

Ingredients	Weight[2]	Cal[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Granola[4]	25	124	0	3	16	5
Soya Protein[5]	15	56	0	14	0	0
Lemon	50	13	45	0	5	0
Fresh Fruit	100	40	90	0	5	0
Corn Oil	100	884	0	0	0	100
Avocado[6]	100	171	74	2	4	17
Breakfast Total	390	1287	209	19	30	122
Baked Potatoes	50	47	38	1	10	0
Soyameat[7]	100	206	70	9	3	18
Dressing[8]	150	736	56	3	8	83
Dinner Total	300	990	164	13	21	101
Amer. Process Cheese[9]	50	194	22	11	2	16
Heavy Whipping Cream	130	458	74	3	4	49
Fruit Cocktail	50	36	41	0	10	0
Supper Total	230	688	137	14	16	65
TOTAL	920	2965	510	46	67	288

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Kellogg Company, Battle Creek, Michigan

[5]Fearn Soya Foods, Melrose Park, Illinois.

[6]Fuerte variety (Calavo), California.

[7]Soyameat, Chicken style, Worthington Foods, Worthington, Ohio.

[8]For composition of the dressing, see Appendix D.

[9]Food Club, Meijer, Inc., Grand Rapids, Michigan.

APPENDIX D

Composition of the Dressing used in the high fat diets during Experiment I.

Ingredient	Weight[1]	Cal.[2]	Water[1]	Protein[1]	CHO[1]	Fat[1]
Avocado[3]	100	171	74	2	4	17
Soyamel	50	235	3	11	23	11
Corn Oil	300	2532	0	0	0	300
Vinegar	50	7	47	0	3	0
Water	100	0	100	0	0	0
TOTAL	600	2945	224	13	30	328
Dressing[5]	100	491	37	2	5	55

[1]Weight in grams.

[2]Calories in kcal.

[3]Fuerte variety (Calavo), California.

[4]Fortified Soyamel, Worthington Foods, Worthington, Ohio.

[5]Composition per 100 g dressing.

APPENDIX E

Composition of the high carbohydrate control diet for Experiment II (approx. 5% of calories from protein and 85% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Orange	200	98	172	2	24	0
Grapefruit	100	40	88	1	10	0
Lemon	50	14	45	1	4	0
Apple	260	156	218	1	36	2
Banana	300	255	227	3	65	1
Dried Apple	185	509	44	2	127	3
Soya Protein[4]	3	12	0	3	0	0
Breakfast Total	1098	1084	794	13	266	6
Corn Oil	10	88	0	0	0	10
Sweet Potatoes[5]	250	210	190	4	55	1
Minute Rice[5]	50	194	35	4	41	0
Baked Potatoes	200	186	150	5	42	0
Jelly	150	450	44	0	112	0
Dinner Total	660	1128	419	13	250	11
Vegetable Soup[5]	305	201	259	4	37	6
Apple	200	120	168	0	30	1
Orange	100	49	86	1	12	0
Bread	26	59	9	3	12	0
Margarine	10	72	2	0	0	8
Jam	24	72	6	0	18	0
Supper Total	665	573	530	8	109	15
TOTAL	2423	2785	1743	34	625	32

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Fearn Soya Foods, Melrose Park, Illinois.

[5]Food Club, Meijer, Inc., Grand Rapids, Michigan.

APPENDIX F

Composition of the Fat I diet for Experiment II (approx. 5% of calories from protein and 6% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Heavy Whipping Cream	250	945	140	5	6	100
Soya Protein[4]	6	24	0	6	0	0
Water-canned Fruit	125	39	114	1	9	0
TOTAL	381	254	1008	12	15	100

[1]The same mixture was eaten three times a day, and portions were adjusted to the subjects' individual caloric requirements.

[2]Weight in grams.

[3]Calories in kcal.

[4]Fearn Soya Foods, Melrose Park, Illinois.

APPENDIX G

Composition of the Fat II diet for Experiment II (approx. 5% of calories from protein and 6% from carbohyerates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Avocado[4]	200	342	148	4	8	34
Corn Oil	70	619	0	0	0	70
Lemon	50	14	45	1	4	0
Soya Protein[5]	8	32	0	8	0	0
Breakfast Total	328	1007	193	13	12	104
Canned Carrots[6]	50	16	47	0	4	0
Dressing[7]	241	839	133	6	10	89
Soyameat[8]	70	144	49	6	2	13
Dinner Total[9]	361	999	229	12	16	102
TOTAL	1050	3005	651	37	44	308

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Fuerte variety (Calavo), California.

[5]Fearn Soya Foods, Melrose Park, Illinois.

[6]Food Club, Meijer, Inc., Grand Rapids, Michigan.

[7]For composition of the dressing, see Appendix H.

[8]Soyameat, Chicken style, Worthington Foods, Worthington, Ohio.

[9]This meal was used for both dinner and supper.

APPENDIX H

Composition of the Dressing used in the Fat II diet during Experiment II.

Ingredients	Weight[1]	Cal.[2]	Water[1]	Protein[1]	CHO[1]	Fat[1]
Avocado[3]	150	257	111	3	6	26
Almonds	25	150	1	5	4	14
Soya Protein[4]	1	4	0	1	0	0
Lemon	50	13	45	0	5	0
Corn Oil	100	884	0	0	0	100
Water	50	0	50	0	0	0
TOTAL	376	1308	207	9	15	140
Dressing[5]	100	348	55	2.4	4	37

[1]Weight in grams.

[2]Calories in kcal.

[3]Fuerte variety (Calavo), California.

[4]Fearn Soya Foods, Melrose Park, Illinois.

[5]Composition per 100 g dressing.

APPENDIX I

Composition of the high carbohydrate control diet for Experiment III (approx. 15% of calories from protein and 60% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Orange	100	51	85	1	12	0
Apple	100	60	84	0	14	1
Lemon	50	13	45	1	4	0
Grapefruit	200	82	176	1	21	0
Banana	100	85	76	1	22	0
Granola[4]	50	230	3	6	34	9
Soyamel[5]	50	247	2	9	27	13
Soya Protein[6]	15	56	0	14	0	0
Breakfast Total	665	824	471	33	134	23
Baked Potatoes	200	186	150	5	41	0
Soyameat[7]	70	162	39	13	8	9
Soybeans[8]	100	150	66	14	14	4
Canned Pears[9]	400	272	328	0	72	0
Cool Whip[10]	50	154	28	0	11	11
Dinner Total	820	924	611	32	146	24
Vegetable Soup[9]	305	200	259	4	36	6
Bread	57	140	14	6	27	2
Peanut Butter	30	174	1	8	5	15
Soyameat[11]	70	148	43	12	7	8
Apple	200	120	168	0	28	2
Orange	100	51	85	1	12	0
Waffer Snack[12]	50	150	18	2	26	4
Supper Total	812	983	588	33	141	37
TOTAL	2297	2731	1670	98	421	84

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams. [3]Calories in kcal. [4]Kellogg Company, Battle Creek, Mi.

[5]Fortified Soyamel, Worthington Foods, Worthington, Ohio.

[6]Fearn Soya Foods, Melrose Park, Illinois.

[7]Soyameat, Salisbury Steak style, Worthington.

[8]Soybeans, Boston Bean style, Loma Linda Foods, Riverside, CA.

[9]Food Club, Meijer, Inc., Grand Rapids, Michigan.

[10]General Foods, White Plains, New York.

[11]Soyameat, Wham (ham style slices), Worthington.

[12]Nutty Bars, Little Debbie, McKee Baking Company, Collegedale, Tenn.

APPENDIX J

Composition of the Fat I diet for Experiment III (approx. 15% of calories from protein and 5% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Eggs	100	216	68	13	1	17
Amer. Process Cheese[4]	57	210	40	12	2	18
Sour Cream[4]	225	468	162	8	8	47
Breakfast Total	382	894	270	33	11	82
Canned Carrots[4]	65	20	60	0	5	0
Butter	45	324	14	1	1	37
Meat[5]	80	190	44	28	0	8
Sour Cream[4]	113	234	81	4	4	24
Dinner Total[6]	303	768	199	33	10	69
TOTAL	988	2430	668	99	31	220

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Food Club, Meijer, Inc., Grand Rapids, Michigan.

[5]Beef bottom round (lean and trimmed).

[6]This meal was used for both dinner and supper.

APPENDIX K

Composition of the Fat II diet for Experiment III (approx. 15% of calories from protein and 5% from carbohydrates).[1]

Ingredients	Weight[2]	Cal.[3]	Water[2]	Protein[2]	CHO[2]	Fat[2]
Avocado[4]	50	84	37	1	2	8
Soyameat[5]	225	446	153	29	7	36
Almonds	10	60	0	2	2	5
Mayonaise[6]	40	286	9	0	0	31
Breakfast Total	325	876	199	32	11	80
Soyameat[7]	340	700	240	30	10	60
Avocado[4]	50	84	37	1	2	8
Mayonaise[6]	15	109	3	0	0	12
Dinner Total	405	893	280	31	12	80
TOTAL	1135	2662	759	94	35	240

[1]The total caloric intake was adjusted to the subjects' individual requirements by varying the amount of breakfast.

[2]Weight in grams.

[3]Calories in kcal.

[4]Fuerte variety (Calavo), California.

[5]Chicken style slices, Worthington Foods, Worthington, Ohio.

[6]Kraft Foods, Chicago, Illinois.

[7]Chicken style pieces, Worthington.

[8]This meal was used for both dinner and supper.

APPENDIX L

The content of EAA in the high carbohydrate control diet for Experiment II, calculated from the diet composition given in Appendix E.[1] (All values are in g.)

Protein Source	N	Ile.	Leu.	Lys.	TSAA[2]	TAAA[3]	Thr.	Thp.	Val.
Apple	0.48	0.11	0.19	0.18	0.06	0.12	0.11	0.028	0.12
Soya Protein	0.53	0.11	0.14	0.17	0.20	0.23	0.11	0.039	0.14
Banana	0.48	0.09	0.12	0.12	0.14	0.20	0.10	0.035	0.12
Sweet Potato	0.64	0.15	0.22	0.14	0.11	0.25	0.15	0.068	0.31
Potato	0.80	0.19	0.30	0.23	0.09	0.34	0.19	0.082	0.26
Rice	0.67	0.16	0.35	0.16	0.14	0.36	0.16	0.052	0.23
Vegetable Soup[4]	0.64	0.14	0.25	0.20	0.08	0.26	0.15	0.047	0.19
Citrus Fruit	0.80	0.14	0.14	0.26	0.14	0.29	0.08	0.035	0.19
Bread	0.53	0.12	0.20	0.08	0.12	0.24	0.10	0.036	0.14
TOTAL	5.57	1.12	1.91	1.54	1.08	2.29	1.15	0.42	1.70
Requirement[5]	6.37	0.70	1.10	0.80	1.01	1.10	0.50	0.25	0.80

[1]The AA composition is based on the FAO tables (Italy, 1970).

[2]Total S-containing AA (Methionine and Cystine).

[3]Total aromatic AA (Phenylalanine and Tyrosine).

[4]Based on the mean of the major constituents.

[5]From Table 6, p. 40, column 8 of "Improvement of protein nutriture" (NAS, Washington, DC., 1974). These figures are for a 70 kg man and are the highest values required for any subject in the experiment.

APPENDIX M

The content of EAA in the Fat I diet for Experiment II, calculated from the diet composition given in Appendix F.[1]

Protein Source	N	Ile.	Leu.	Lys.	TSAA[2]	TAAA[3]	Thr.	Thp.	Val.
Cream	0.78	0.31	0.61	0.35	0.16	0.64	0.22	0.071	0.36
Soya Protein	1.01	0.28	0.49	0.40	0.16	0.51	0.24	0.081	0.30
Canned Pears	0.16	0.03	0.04	0.03	0.02	0.06	0.03	0.009	0.04
Sum	1.95	0.62	1.14	0.78	0.34	1.21	0.49	0.161	0.70
TOTAL[4]	5.85	1.86	3.42	2.34	1.02	3.63	1.47	0.48	2.10
Requirement[5]	6.37	0.70	1.10	0.80	1.01	1.10	0.50	0.25	0.80

[1]The AA composition is based on the FAO tables (Italy, 1970).

[2]Total S-containing AA (Methionine and Cystine).

[3]Total aromatic AA (Phenylalanine and Tyrosine).

[4]The total intake per day is calculated by multiplying the sum by three, since identical meals were fed three times a day.

[5]From Table 6, p. 40, column 8 of "Improvement of protein nutriture" (NAS, Washington, DC, 1974). These figures are for a 70 kg man, and are the highest values required for any subject in the experiment.

APPENDIX N

The content of EAA in the Fat II diet for Experiment II, calculated from the diet composition given in Appendix G.[1]

Protein Source	N	Ile.	Leu.	Lys.	TSAA[2]	TAAA[3]	Thr.	Thp.	Val.
Avocado	1.37	0.29	0.47	0.37	0.34	0.50	1.12	0.170	0.40
Lemon	0.20	0.04	0.03	0.07	0.03	0.07	0.02	0.009	0.05
Soya Protein	1.56	0.44	0.76	0.62	0.25	0.79	0.38	0.125	0.47
Almonds	1.24	0.22	0.38	0.31	0.19	0.42	0.22	0.087	0.28
Soyameat	2.10	0.60	1.02	0.84	0.34	1.06	0.51	0.168	0.63
TOTAL	6.47	1.59	2.66	2.21	1.15	2.84	2.25	0.56	1.83
Requirement[4]	6.37	0.70	1.10	0.80	1.01	1.10	0.50	0.25	0.80

[1]The AA composition is based on the FAO tables (Italy, 1970).

[2]Total S-containing AA (Methionine and Cystine).

[3]Total aromatic AA (Phenylalanine and Tyrosine).

[4]From Table 6, p. 40, column 8 of "Improvement of protein nutriture" (NAS, Washington, DC, 1974). These figures are for a 70 kg man, and are the highest values required for any subject in the experiment.