The regulatory, informational, and immunomodulatory roles of fat fuels

Eric A Newsholme, Philip Calder, and Parveen Yaqoob

ABSTRACT Fat oxidation provides a fuel for many tissues and it provides an important signal to decrease glucose utilization and oxidation in muscle and so conserve glucose for essential organs such as the brain. The control of fatty acid oxidation is achieved in part through its plasma concentrations, which may be precisely controlled by the triacylglycerol–fatty acid substrate cycle, which can also, if oxidation is taken into account, be viewed as a branch point in this important pathway. Branch points may provide precision in regulation if one of the fluxes at the branch is low compared with the other flux. Both branch points and substrate cycles are energetically expensive and may account for some of the increases in energy expenditure in conditions of injury, burns, and sepsis and in the postexercise condition. Fatty acids, through effects on plasma free tryptophan concentrations and hence 5-hydroxytryptamine concentrations in the brain, may play a role in central fatigue. Polyunsaturated fatty acids are claimed to have immunosuppressive properties. Work has been done to provide a biochemical analysis of how they might influence some functions of cells of the immune system. Am J Clin Nutr 1993;57(suppl):738S–51S.

KEY WORDS Triacylglycerol, fatty acids, ketone bodies, substrate cycles, energy expenditure, regulation, immunosuppressive, polyunsaturated fatty acids, fatigue

Introduction

The wealth of information about metabolism that has accrued over the past 50 years, the large number of wall charts describing increasingly complex metabolic pathways, and the rapid development of molecular biology have led to the view that most of general metabolism is understood and further work will provide only trivial information. Consequently, there is a feeling that current investigations in the metabolic and nutritional fields are not only old-fashioned but are probably a waste of time, effort, and money.

The information provided in this paper will attempt to show the naiveté of such views. Work on the effects of the metabolism of fat and its role in cellular nutrition over the past 30 years has indicated its importance in a wide range of biochemical, physiological, and immunological areas, including the following:

- control of the blood sugar concentrations;
- control of glycogen synthesis after exercise;
- control of body weight;
- fueling of the immune system and the significance of these fuels for immune-cell function;
- biochemical causes of physical and mental fatigue;
- mechanisms by which fatty acids can influence the effectiveness of the immune system.

To attempt to understand the importance of fat it is necessary to know where it is used. Thus, two basic questions that are fundamental to cellular nutrition are, how is it possible to identify which fuels particular cells can use in vivo, and what is the maximum capacity (i.e., the maximum possible rate at which the fuel can be used by the tissue) for the use of each fuel? One way to answer these questions requires some knowledge of metabolic-control logic: a brief introduction to this topic is given below.

Metabolic-control logic 1: structure of a biochemical pathway or process

Near-equilibrium and nonequilibrium reactions

Reactions in a metabolic pathway can be divided into two classes: those that are very close to equilibrium (near-equilibrium) and those that are far removed from equilibrium (nonequilibrium). For a nonequilibrium reaction, the rate of the reverse component ($V_r$) of the reaction is very much less than the rate of the forward component ($V_f$), for example:

$$A \xrightarrow{10.01} B$$

With a near-equilibrium reaction, the rates of the forward and the reverse components of the reaction are much greater than the overall net rate and are similar to one another, for example:

$$A \xrightarrow{100} B$$

The equilibrium nature of a reaction can be decided on the basis of the free-energy change of the reaction ($\Delta G$), which can be calculated from a knowledge of the ratio of the concentrations of product to the concentration of substrate in the living cell or tissue, which is known as the mass action ratio, and the equilibrium constant, $K_{eq}$, of the reaction as follows:

$$\Delta G = -RT \ln K_{eq} + RT \ln \left[ \frac{\text{product}}{\text{substrate}} \right]$$

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When \( \Delta G \) is > 4.2 kJ/mol (> 1.0 kcal/mol) the reaction is considered to be nonequilibrium, and when it is < 1.0 kcal/mol, the reaction is considered to be near equilibrium (1). There is a “grey” area: for values close to 1 kcal/mol, it is difficult to decide on the equilibrium nature.

The flux-generating reaction

If an enzyme catalyzes a nonequilibrium reaction in a biochemical process and it approaches saturation with its pathway substrate (the substrate that represents the flow of matter through the pathway), so that the catalytic rate is independent of that substrate concentration, the reaction can be regarded as the flux-generating step for the pathway. In other words, in the steady state, this reaction initiates a flux to which all the other reactions in the pathway must adjust. (Such a reaction must approach saturation with its pathway substrate because, if it did not, as the reaction proceeded the substrate concentration would decrease and this would decrease the rate of the reaction and hence the flux through the pathway: a steady state would then be impossible.) One important development from the concept of a flux-generating step is that it provides a physiologically useful definition of a metabolic pathway and it provides a structure for the pathway that is particularly important in understanding how the flux is controlled. A pathway is defined as a series of reactions that is initiated by a flux-generating step and ends with the loss of end product to a metabolic sink (storage form) or to the environment, or ends in a reaction that precedes another flux-generating step. This definition enhances our understanding of how the control of changes in flux through a pathway may be brought about, and it allows a more objective interpretation of the function of a pathway, and it allows us to define limitations in our understanding of these functions. This approach will be used for fatty acid metabolism in this paper. An important question arises in cell physiology and cell nutrition: how is it possible to measure the capacity of a cell to use a given fuel—especially if the interest is in a wide variety of cells or tissues? Several methods for doing this have been reviewed elsewhere (2); here, attention is focused on the maximum catalytic activity principle for measuring maximum flux through pathways.

Use of maximum activities of enzymes as quantitative indexes of maximum flux through metabolic pathways

Advantages and requirements

The advantage of a near-equilibrium reaction, in a metabolic pathway in vivo, is that the reaction may be very sensitive to small changes in concentrations of substrate, co-substrate or co-product. Consequently, large changes in flux can be transmitted through such a reaction without any requirement for complex regulatory properties (3). In general, this means that the activity of the enzyme can be measured relatively easily in crude extracts of the tissue. The ease of this assay has been used by some investigators as the only criterion for the selection of an enzyme whose maximum catalytic activity may provide useful information on maximum flux through a metabolic pathway. However, metabolic logic tells us that maximum activities of these enzymes cannot be used as quantitative indexes of flux (4), but, despite this, they are, even in 1992, still being used either unwittingly or unwittingly in attempts to provide such information. Failure to use this knowledge may lead, at best, to misinterpretation of data and, at worst, to serious misapplication of experimental effort and financial resources.

Enzymes that catalyze nonequilibrium reactions in a metabolic pathway provide directionality in that pathway and may be subject to allosteric control. Indeed, the control mechanisms may be complex. This means that knowledge of such control mechanisms must be available before a satisfactory assay method for measurement of the maximum activity can be developed; hence, knowledge of metabolic control is necessary to enable the maximum enzyme activity to be adequately assayed in crude extracts of the tissue.

At least two requirements must be met before an enzyme activity can be used to provide quantitative information:

- establishing which enzymes in the pathway catalyze nonequilibrium reactions (see above);
- demonstrating experimentally that, at least in some conditions and in some cells, the maximum activities of such enzymes in vitro do indeed indicate quantitatively the maximum flux through a reaction. This is done by comparison of the in vitro enzyme activity with the measured or calculated maximum flux through the pathway.

Enzyme activities as indicators of the capacity of major energy-providing pathways in muscle and in some immune cells

Muscle: Systematic studies in the 1970s on the maximum activities of key enzymes for the utilization of carbohydrate, fat, and some amino acids in muscle provided information on the types of fuel used by different muscles and their maximum contribution to adenosine triphosphate (ATP) formation to support contractile activity. A systematic and comprehensive analysis could then be made of the fuels used by different muscles from different animals across the animal kingdom. Of particular importance, use of this approach provided information on the maximum anaerobic and aerobic capacities of muscle (Table 1) (5) and on which muscles can utilize fatty acid and ketone bodies as fuels, which is important in understanding the glucose–fatty acid and glucose–fatty acid–ketone body cycles. Furthermore, this knowledge, together with quantitative information on amounts of fuels stored in the body and the communication

<table>
<thead>
<tr>
<th>Group</th>
<th>Anaerobic glycolysis</th>
<th>Oxidation via Krebs cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g} )</td>
<td></td>
</tr>
<tr>
<td>Untrained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>104</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>87</td>
<td>16</td>
</tr>
<tr>
<td>Medium-trained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>91</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>89</td>
<td>19</td>
</tr>
<tr>
<td>Well-trained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
<td>26</td>
</tr>
<tr>
<td>Female</td>
<td>61</td>
<td>29</td>
</tr>
</tbody>
</table>

* Maximum rates of ATP formation are calculated as follows: for anaerobic glycolysis, 6-phosphofructokinase activity is multiplied by 3; for oxidation by the Krebs cycle, oxoglutarate dehydrogenase activity is multiplied by 18. Enzyme activity data are from reference 5.
TABLE 2
Maximum activities of 3-hydroxybutyrate dehydrogenase, 3-oxoacid CoA transferase, and carnitine palmitoyltransferase in muscles of teleosts and elasmobranchs*

<table>
<thead>
<tr>
<th>Animal and muscle</th>
<th>3-Hydroxybutyrate dehydrogenase</th>
<th>3-Oxoad CoA transferase</th>
<th>Carnitine palmitoyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol·min⁻¹·g fresh tissue⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>&lt;0.01</td>
<td>13.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Red</td>
<td>&lt;0.01</td>
<td>8.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Bass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>&lt;0.01</td>
<td>17.6</td>
<td>0.60</td>
</tr>
<tr>
<td>Red</td>
<td>&lt;0.01</td>
<td>18.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Mackerel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>&lt;0.01</td>
<td>13.3</td>
<td>0.40</td>
</tr>
<tr>
<td>Red</td>
<td>&lt;0.01</td>
<td>11.1</td>
<td>0.60</td>
</tr>
<tr>
<td>Dogfish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>1.2</td>
<td>9.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Red</td>
<td>1.3</td>
<td>15.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Branchial</td>
<td>0.65</td>
<td>7.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ray</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.72</td>
<td>37.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Red</td>
<td>0.37</td>
<td>27.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Spur dog</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.21</td>
<td>9.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Red</td>
<td>0.64</td>
<td>6.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smooth hound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.27</td>
<td>11.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Red</td>
<td>0.84</td>
<td>22.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Data from reference 6.

between tissues provided by fuels, has led to new ideas on causes of fatigue and limitations in performance in Olympic events and how these might be modified by nutritional changes (discussed below).

The evolution of fatty acids and ketone bodies as fuels: a study based on enzyme activities. An interesting development that may reflect the evolution of fatty acids and ketone bodies was made by using the maximum-enzyme-activity approach. For ketone body utilization the activity of 3-oxo-acid CoA transferase cannot provide quantitative information about flux through the ketone body pathway, but it provides useful qualitative information.

The activities of 3-oxo acid CoA-transferase were found to be similar in the muscles of three teleost fish (mackerel, plaice, and bass) to those in the muscles of four elasmobranchs (smooth hound, spur dog, ray, and dogfish), which are more primitive fish (Table 2). However, carnitine palmitoyltransferase activity was not detectable in any of the muscles of the four elasmobranchs investigated, whereas it was present in those of the teleosts (Table 2). Carnitine palmitoyltransferase activity was present in the livers of the elasmobranchs investigated: 0.04 and 0.07 µmol·min⁻¹·g⁻¹ for dogfish and ray, respectively. These results suggested that fatty acids are not important as a fat fuel in the muscle of elasmobranchs. For this reason, the concentrations of ketone bodies, fatty acids, and glycerol were measured in the blood of teleosts and elasmobranchs during starvation (6).

The blood concentration of nonesterified fatty acids in bass increased after 40 and 100 d starvation and the concentrations were similar to those in rats after 2 d and in humans after 6 d starvation. The increase in the blood concentration of glycerol in bass suggests that the increased nonesterified fatty acids concentration represents increased mobilization from the distinct adipose tissue stores of triacylglycerols present in this fish. Despite this increase in nonesterified fatty acids, there is no statistically significant increase in the concentration of acetoacetate in bass during starvation, and the total ketone-body concentration remained very low (Table 3). However, in the dogfish the nonesterified fatty acid concentration decreased during starvation, whereas that of ketone bodies increased at each time interval investigated; at 150 d the ketone body concentration increased by 28-fold to reach a value of 2.28 mmol/L, which is similar to that observed in the rat after 96 h starvation. Furthermore, as in the mammals, the largest concentration increase occurs in 3-hydroxybutyrate (0.01–1.86 mmol/L), so the hydroxybutyrate–acetoacetate concentration ratio increases markedly. These findings suggest that in the dogfish, hepatic triacylglycerol stores

TABLE 3
Concentrations of glucose, acetoacetate, hydroxybutyrate, and nonesterified fatty acids in blood or plasma of fed and unfed bass and dogfish*

<table>
<thead>
<tr>
<th>Animal and time after capture</th>
<th>Glucose</th>
<th>Acetoacetate</th>
<th>3-Hydroxybutyrate</th>
<th>Nonesterified fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>Starved</td>
<td>Fed</td>
<td>Starved</td>
</tr>
<tr>
<td>Dogfish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>1.42</td>
<td>—</td>
<td>0.07</td>
<td>—</td>
</tr>
<tr>
<td>40 d</td>
<td>0.49</td>
<td>0.55</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>100 d</td>
<td>0.62</td>
<td>0.41</td>
<td>0.11</td>
<td>0.38</td>
</tr>
<tr>
<td>150 d</td>
<td>0.50</td>
<td>0.37</td>
<td>0.07</td>
<td>0.42</td>
</tr>
<tr>
<td>Bass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>7.39</td>
<td>—</td>
<td>0.04</td>
<td>—</td>
</tr>
<tr>
<td>40 d</td>
<td>2.61</td>
<td>2.63</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>100 d</td>
<td>3.20</td>
<td>1.91</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>150 d</td>
<td>2.95</td>
<td>1.06</td>
<td>0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Data from reference 6.
are mobilized not as nonesterified fatty acids but as ketone bodies. This suggestion is supported by the inability to detect glycerol in the blood, since it is probable that the glycerol that is produced from hepatic lipolysis will be phosphorylated within the liver.

The reason for the dependence of the dogfish (and presumably all elasmobranchs) on ketone bodies as the major fat fuel during starvation is not known. It is possible that, for some unknown reason, nonesterified fatty acids cannot be released from the liver. Because nonesterified fatty acids are not very soluble in aqueous medium, physiologically meaningful quantities of nonesterified fatty acids can only be transported if a transport protein, such as albumin, is present in the blood. Albumin is not present in the blood of the elasmobranchs, but it is present in that of most teleosts, including bass. Once a transport protein for nonesterified fatty acids is present in the blood, triacylglycerols can be mobilized as nonesterified fatty acids, and the dependence on ketone bodies is removed. Thus, the teleosts may represent an important evolutionary position in metabolism because they are the first group of animals that have developed a specific storage tissue for triacylglycerols and the ability to mobilize and transport nonesterified fatty acids.

The apparent reluctance of teleosts to convert nonesterified fatty acids into ketone bodies during starvation is in marked contrast to the situation in higher animals. This poses interesting questions about why both nonesterified fatty acids and ketone bodies need to be utilized in higher animals during starvation, and at what stage in the evolutionary development both nonesterified fatty acids and ketone bodies are available in the blood during starvation. The increasing size of the brain in relation to body size and the need to restrict glucose formation from amino acids in order to conserve body protein may be a possible answer because ketone bodies, but not nonesterified fatty acids, are utilized in preference to glucose by the brain of higher animals (2). Ketone bodies may have other important roles in higher animals.

Immune cells. More recently, the maximum-enzyme-activity approach has been applied to lymphocytes, macrophages, and endothelial cells, which has provided, for the first time, evidence that these cells can use glutamine and/or long-chain fatty acids for energy formation and, indeed, that these fuels could be quantitatively more important than glucose (7–9). Before this, glucose was considered the major, if not the only, fuel used by lymphocytes.

The maximum activities of carnitine-palmitoyl transferase in lymphoid tissue are low compared with those in skeletal muscle and heart (7–9). Nonetheless, they suggest that there is a sufficient capacity for fatty acid oxidation that it could provide a significant proportion of the ATP used by these cells. Indeed, it is suggested that one of the major fuels for the lymphocyte and macrophage, even in the fed condition, is fatty acid. Neither the rate of oleate utilization nor the rate of O2 uptake is affected by increasing the oleate concentration from 0.5 to 1.5 mmol/L; this is in complete contrast with the situation known to exist for other tissues (including heart, kidney, and skeletal muscle), in which the rate of fatty acid oxidation increases markedly as the concentration increases above 0.5 mmol/L. Furthermore, the rate of uptake of fatty acid by the heart and skeletal muscle exhibits a threshold effect: below about 0.35 mmol/L very little or no fatty acid uptake occurs (8, 9). The plasma fatty acid concentration in the normal, fed animal (rat or man) is ~0.5 mmol/L and increases toward 2 mmol/L in prolonged starvation (2). This leads to the important conclusion that fatty acids may be utilized and oxidized by lymphocytes and macrophages even in the fed state and that starvation makes no difference. The significance of this may be that it may enable fatty acids to be removed from the bloodstream in the fed animal to support some, and possibly a large proportion, of the energy demands of the lymphocyte.

The regulatory effects of fatty acids

The glucose-fatty acid cycle

For fat to be oxidized by muscle and other tissues it has to be mobilized from adipose tissue as fatty acids. These are transported bound to albumin in the plasma, and their plasma concentration, in part, plays a role in control of the rate of oxidation of fatty acids in muscle. However, fatty acids not only play a role as a fuel but also as a regulator of glucose utilization and oxidation.

The concept of the glucose-fatty acid cycle was put forward to explain the reciprocal relationship between the rates of oxidation of glucose and fatty acids by muscle (2, 10). Although some features of the cycle have been modified since that time, there is now considerable evidence to support the important proposal that, under conditions of "carbohydrate stress" (defined as when the glycogen store in the liver is depleted), fatty acids are mobilized from adipose tissue so that their rate of oxidation by muscle increases and this, in turn, decreases the rate of glucose utilization (2). Conversely, when the carbohydrate stress is removed (e.g., by refeding a starved subject), the rate of fatty acid release by adipose tissue is reduced, decreasing the rate of fatty acid oxidation, so that the rate of glucose utilization by the muscle increases. These responses serve to stabilize the blood glucose concentration (Fig 1).

The regulatory effect of fatty acid on glucose utilization can be seen as a logical necessity when the small reserves of carbohydrate are taken into account together with the facts that some tissues have an obligatory requirement for glucose and that there is a problem in too much glucose being provided by gluconeogenesis for oxidation. The lipid reserves in the average human subject are 20 times higher than carbohydrate reserves (2). The

![FIG 1. The glucose-fatty acid cycle. Note that a change in the peripheral blood glucose concentration is less important than are changes in the glucose concentration in hepatic portal blood in eliciting insulin release from the pancreas because glucose absorption from the intestine is accompanied by secretion of duodenal hormones. (FFA, free fatty acids.)](https://academic.oup.com/ajcn/article-abstract/57/5/738S/4715955)
reason for this marked preference for lipid as a reserve fuel, which is not unique to humans but is found in most animals, is that it is approximately nine times more efficient than carbohydrate as a storage fuel.

Lever contains the only store of glycogen that can be broken down into glucose and released into the bloodstream for use by other tissues (muscle glycogen is used solely within the muscle). However, this quantity of stored carbohydrate (~100 g in the adult) is very small in relation to the glucose requirement of the tissues. At rest, the total glucose requirement of the major carbohydrate-utilizing tissues of the body (brain, kidney, heart, and muscle) is >300 g/d, which is normally met by the dietary intake of carbohydrate. Furthermore, the amount of carbohydrate stored in the liver of a child is very small in relation to the child’s demand for glucose. In the early period of starvation, liver glycogen is broken down to provide glucose for the tissues, and this lasts for ~24 h. Because some tissues can oxidize fatty acids as well as glucose, the mobilization of fatty acids from the adipose-tissue triacylglycerol store probably begins during the overnight fast and increases, particularly if breakfast is missed. In this way, some of the glucose derived from liver glycogen will be preserved for tissues that must oxidize glucose (eg. the cells of the brain).

Sustained exercise is another condition for which there is good evidence that skeletal muscle utilizes fatty acids. The rate of mobilization of fatty acids will depend on the intensity of the exercise, its duration, and probably the extent of carbohydrate stress (liver and possibly muscle glycogen concentrations). High plasma concentrations of fatty acids may be involved not only in providing fuel and as a regulator of glucose utilization but, in addition, may be involved as a signal for central fatigue in exercise and perhaps other conditions (see below).

**Situations in which the blood glucose concentration is maintained by the control cycles**

Some physiological and pathological conditions in which the glucose–fatty acid cycle is of fundamental importance are given below (see ref 2).

**Sustained exercise.** Since the liver glycogen reserves are partially depleted by the overnight fast, even moderate exercise (eg. walking or jogging) before breakfast causes considerable mobilization of fatty acids. However, prolonged exercise increases fatty acid mobilization under all conditions. There is evidence that after ~30 min of exercise at 60–70% of VO2max, mobilization of fatty acids from adipose tissue increases (2). However, despite increased mobilization of fatty acids, the plasma concentration of fatty acids may be only slightly increased because the flow of blood through the muscle and the utilization of the blood by muscle are also increased. It is possible that the fatty acid concentration increases markedly only when the muscle (and liver) glycogen stores become depleted or when the rate of fatty acid oxidation by muscle is lowered because of the intermittent nature of the exercise that occurs in games such as soccer, rugby, tennis, and squash, for example. The importance of this in causing fatigue is discussed below.

**Starvation.** In the absence of exercise, the overnight fast results in a small increase in the rate of fatty acid mobilization. Glycogen in the liver provides most of the glucose requirements until a carbohydrate breakfast restores the dietary supply of glucose. However, when breakfast is not eaten, so that the fast is extended to 12–18 h, there is a marked increase in the rate of mobilization of fatty acids and in the operation of the glucose–fatty acid cycle, which prevents a serious fall in glucose concentration.

**Refeeding after starvation.** During starvation, glucose is conserved. If starvation is terminated with a high-carbohydrate meal, failure to reverse this restriction could result in a massive elevation in the blood glucose concentration. This could result in loss of glucose in the urine with the attendant problems of dehydration and loss of ions from the blood.

**Stress.** Increasing the plasma fatty acid concentration in stress would, through the operation of the glucose–fatty acid cycle, reduce the likelihood of serious hypoglycaemia if “fight or flight” took place. Since this type of “exercise” is likely to be anaerobic, fatty acids will not restrict the rate of glycolysis and, hence, of energy generation in muscle under these conditions.

**Hypoglycemia.** There are several pathological conditions in which the blood glucose concentration is below normal and, consequently, the concentration of fatty acids is elevated. In these conditions, the operation of the cycle prevents a catastrophic fall in the blood glucose concentration. In some conditions, particularly in infants and young children and after prolonged exercise in adults, the concentration of ketone bodies also increases, which increases the inhibitory effect on glucose utilization (2).

**Obesity.** In obesity, the amount of adipose tissue is increased. An increased amount of adipose tissue results in a greater rate of fatty acid mobilization than expected for the given concentrations of the hormones insulin and catecholamines. This may be due to a specific decrease in the sensitivity of adipose tissue to insulin or it may be caused simply by a “mass” effect of the adipose tissue. This greater rate of fatty acid mobilization in turn results in a greater rate of fatty acid oxidation by muscle and hence lowers the rate of glucose utilization and oxidation, which will cause an increase in the plasma glucose concentration and hence a greater increase in the insulin concentration required to lower that of glucose. The fact that more insulin is required to regulate the blood glucose concentration is termed insulin resistance—a condition that characterizes the obese state.

**Trauma, surgery, sepsis, and burns.** Trauma, whether accidental or as a result of the surgeon’s knife, sepsis, or burns all result in an increased rate of mobilization of fatty acids from adipose tissue and elevated plasma fatty acid concentrations. Hence, the glucose–fatty acid cycle operates under these conditions. One advantage of this is to conserve glucose for the brain because trauma may restrict food intake and, in primitive humans, would have prevented hunting or gathering. However, high rates of glucose utilization are required for some of the cells of the immune system, for fibroblasts, and for general biosynthesis. The enhanced rate of fatty acid mobilization may be caused by elevated concentrations of catecholamines and glucocorticoids, although cytokines may also be involved.

One very important feature of these conditions is that the mobilization of fatty acids from adipose tissue is resistant to the effects of glucose and insulin. Hence, glucose infusion in such patients does not lower the fatty acid concentration, so it does not result in increased rates of glucose utilization by the major tissues such as muscle: the glucose–fatty acid cycle still operates. Consequently, this extra glucose is converted into storage products, glycogen, and lipid. This in itself might not be a problem, but the biosynthesis of these compounds requires energy, and, therefore, increased rates of oxidation are necessary to provide the ATP required for the biosynthesis. This increased oxidation will result in a greater rate of production of CO2, which may pose a threat to the patient if there is damage to the chest making respiration difficult. Accumulation of CO2 can then easily occur.
resulting in acidosis, which could be fatal (11). This emphasizes the important question of which fuels and how much of such fuels should be provided for patients through parenteral nutrition.

Metabolic-control logic 2: sensitivity in metabolic regulation and the role of the substrate cycle

Sensitivity defined

To understand the suggested role of the glucose-fatty acid substrate cycle, it is necessary to have some knowledge of the concept of sensitivity. Sensitivity in metabolic regulation can be defined as the quantitative relationship between the relative change in enzyme activity and the relative change in concentration of the regulator. (If the concentration of a regulator \(x\) changes by \(\Delta x\), the relative change is \(\Delta x/x\); similarly, if the flux \(J\) changes by \(\Delta J\), the relative change is \(\Delta J/J\). The sensitivity of \(J\) to the change in concentration of \(x\) is given by the ratio \((\Delta J/J)/(\Delta x/x)\), and this sensitivity is indicated by the symbol \(s\) (see Fig. 2). For example, if the concentration of a regulator increases twofold, the question arises, how large an increase in enzyme activity will this produce? The greater the response of enzyme activity to a given increase in regulator concentration, the greater is the sensitivity. To understand more clearly what may be involved in providing sensitivity, it is necessary to begin with the simplest way in which a protein can interact with a small regulator—that is, via equilibrium binding.

Equilibrium binding of a regulator to an enzyme

It is likely that all regulators modify the activity of an enzyme by binding in a reversible manner to a protein. (This protein may not be the immediate “metabolic” enzyme but may be an enzyme involved in an interconversion cycle that controls the target enzyme by covalent modification.) Such binding, which is described as equilibrium binding, will control the activity of the enzyme as follows:

\[ E + R \rightleftharpoons E^*R \]

where \(E\) is the inactive form of the enzyme, \(E^*\) is the active form, and \(R\) is an activator. The asterisk indicates that the binding of the effector molecule \(R\) has changed the conformation of the catalytic site of the enzyme to produce the active form of the enzyme. The normal response of enzyme activity to the binding of the regulator is hyperbolic. The hyperbolic response is the simplest relationship between, for example, a protein and its regulator, a hormone and its receptor, or a neurotransmitter and its receptor. Unfortunately, this response is relatively inefficient for metabolic regulations; for example, a twofold change in regulator concentration will change the enzyme activity by less than twofold (ie, the maximum sensitivity is < unity) (Table 4). This may be difficult to accept when simply observing the steepness of the initial part of a hyperbolic curve. However, it must be appreciated that sensitivity is defined on the basis of relative rather than absolute changes in concentration. Not surprisingly, nature has provided several mechanisms for improving sensitivity in control over that provided by the hyperbolic curve (1-3). One of these that is particularly relevant there is the substrate cycle.

Substrate cycles

It is possible for a reaction that is nonequilibrium in the forward direction of a pathway (ie, \(A \rightarrow B\)) to be opposed by a reaction that is nonequilibrium in the reverse direction of the pathway (ie, \(B \rightarrow A\)). Both reactions must be chemically distinct (different reactions), so they are catalyzed by separate enzymes (Fig 3). A substrate cycle between \(A\) and \(B\) occurs if the two enzymes are simultaneously catalytically active. For every molecule of \(A\) converted to \(B\) and back again to \(A\), chemical energy must be converted to heat, which is lost to the environment.

The role of substrate cycling in the provision of sensitivity and flexibility in metabolic regulation has been discussed in detail in several reviews. It can best be understood when it is appreciated that, in some conditions, an enzyme activity may have to be reduced to values approaching zero. For equilibrium binding, this would require that the concentration of an activator be reduced to almost zero or that of an inhibitor increased to an almost infinite level (due to the relatively ineffec...
TABLE 4
The change in concentration of regulator that is necessary to increase the activity of an enzyme from 10% to 90% of its $V_{\text{max}}$ assuming hyperbolic or sigmoid binding of regulator.

<table>
<thead>
<tr>
<th>Allosteric constant (L)</th>
<th>Concentration of regulator providing 10% of $V_{\text{max}}$ (arbitrary units)</th>
<th>Percent increase in concentration of regulator necessary to increase enzyme activity from 10% to 90% of $V_{\text{max}}$ $\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (hyperbolic)</td>
<td>0.11</td>
<td>8100</td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
<td>9.0</td>
</tr>
<tr>
<td>100</td>
<td>1.20</td>
<td>8.0</td>
</tr>
<tr>
<td>500</td>
<td>2.00</td>
<td>9.8</td>
</tr>
<tr>
<td>1000</td>
<td>2.60</td>
<td>11.5</td>
</tr>
<tr>
<td>10000</td>
<td>5.00</td>
<td>12.6</td>
</tr>
</tbody>
</table>

* Fractional saturation for sigmoid response is calculated according to the simplest model of Monod et al (see ref 2).

When the precise quantitative role of substrate cycles in metabolic control is considered (1, 3).

One advantage of the substrate-cycling mechanism for increasing sensitivity in that the sensitivity can be varied quickly, effectively, and transiently. In addition, when the sensitivity is increased by increased cycling rates, this does not interfere in the basic cellular control mechanism. This change in sensitivity is achieved by varying the ratio of cycling activity is proportional to the ratio of cycling rate to flux. And the rate of cycling, and therefore the sensitivity (as defined above), can be controlled by enzymes. An example of a substrate cycle that is relevant to this chapter is the triglyceride–fatty acid cycle.

The triglyceride–fatty acid cycle

The triglyceride–fatty acid cycle consists of the processes of lipolysis of triglyceride and esterification of fatty acids (Fig 4). This cycle comprises several reactions such that one turn of the cycle results in the hydrolysis of eight molecules of ATP to adenosine diphosphate (ADP) and phosphate. Evidence for the existence of this cycle in adipose tissue has been available for many years: in 1959, it was observed that adrenaline increased the rate of glucose incorporation into triglyceride-glycerol without any change in the rate of fatty acid synthesis (12). This indicates an increase in the rate of this substrate cycle. It has been established that the rate of this cycle is increased in isolated adipose tissue of the rat by both adrenaline and glucagon, and furthermore, that feeding an animal or injection of beta-receptor agonists increases the rate of this cycle in adipose tissue in vivo (13). Furthermore, this increase in cycling rate is inhibited by the beta-blocker propranolol (13).

It is suggested that this increase in cycling rate in vivo improves the sensitivity of the triglyceride–fatty acid cycle to changes in the concentration of hormones such as insulin. Thus, for example, the rate of lipolysis is inhibited and the rate of esterification is increased precisely to accommodate the requirements for energy storage after feeding, after exercise, and for mobilization of fatty acids for fuel for muscle during conditions such as injury, sepsis, burns.

The importance of the triglyceride–fatty acid cycle in increasing the sensitivity of metabolic regulation has recently been established in human white adipose tissue in vivo. Using [1-13C]palmitate and deuterated glycerol, Wolfe and Peters demonstrated a 2.7-fold increase in the rate of triglyceride–fatty acid cycling in response to glucose infusion (14). The rate of this cycle has also been shown to be higher in burn patients and after exercise (15).

In 1976 Newsholme and Crabtree (1) suggested another role for the triglyceride–fatty acid cycle: buffering the intracellular fatty acid concentration in muscle. If the triglyceride lipase activity is increased by, for example, increasing the plasma catecholamine concentration, then the rate of release of fatty acids from stored triglyceride is increased. If these fatty acids are not

![FIG 3](https://example.com/fig3.png)

FIG 3. A hypothetical substrate cycle. In this hypothetical pathway, enzyme $E_5$ catalyzes a key regulatory reaction and, to improve the sensitivity of control at this reaction, a reverse reaction catalyzed by enzyme $E_6$ is present in the tissue, and both enzymes are simultaneously catalytically active. It is proposed that the activities of both enzymes can be increased to increase the rate of substrate cycling and hence can improve sensitivity for metabolic control.

![FIG 4](https://example.com/fig4.png)

FIG 4. The triacylglycerol–fatty acid substrate cycle. Reaction 1 is lipolysis, reaction 2 is fatty acid activation, and reaction 3 is the esterification process.

TABLE 5
Effect of increase in regulator concentration on net flux through a reaction (enzyme activities) controlled by a substrate cycle.

<table>
<thead>
<tr>
<th>Concentration of regulator</th>
<th>$E_2$</th>
<th>$E_5$</th>
<th>Net flux A to B†</th>
<th>Relative increase in flux $U/\text{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>10</td>
<td>9.8</td>
<td>0.2</td>
<td>—</td>
</tr>
<tr>
<td>Fourfold increase</td>
<td>90</td>
<td>9.8</td>
<td>81.2</td>
<td>406 times</td>
</tr>
</tbody>
</table>

* The activities are hypothetical (see Fig 3).
† $E_3 - E_2$. 

by guest
on 25 February 2018
was required, the increase may not be achieved by this system because of the opposition effect. There are two solutions to this problem. One is to provide a sensitive feedback regulatory mechanism to increase the flux through the pathway when required. The other is to increase massively the flux through $J$ and $J_s$. In other words, the highest sensitivity for the flux $J_s$ is achieved when the fluxes $J$ and $J_s$ are much greater than $J_x$. Under these conditions the biosynthetic pathway ($J_s$) does not become seriously limited by changes in precursor concentration during the "deflection" of the flux from $J_s$ to $J_x$ and is therefore most sensitive to the action of regulator $X$.

In nonmathematical terms, high sensitivity is achieved because the rate of the low-flux pathway (that is, $J_s$) can be increased markedly without decreasing significantly the concentration of the metabolic intermediate(s) (eg. $B$) at the branch point of the pathways: a large decrease in the concentration of $B$ would ‘oppose’ the stimulation of the rate in the low-flux pathway (16, 17). Thus the system also serves to stabilize the concentration of $B$.

Glycolysis and glutaminolysis are examples of branched pathways because they provide intermediates that are precursors for biosynthetic pathways, but the fluxes through the biosynthetic pathways are normally very small (Fig 6). However, these fluxes will increase considerably during the synthesis of DNA, RNA, and protein and also during synthesis of phospholipid for new cell membranes. The high rates of glycolysis and glutaminolysis in lymphocytes, macrophages, and tumor cells are, therefore, considered to provide branch-point sensitivity, that is, precision in control, to enable cell division to occur rapidly, effectively, and with minimal cell death. The "inefficient" utilization of these fuels in terms of energy provision is the "cost" that has to be paid for this precision in control (see below). A high rate of glutamine utilization can be maintained only if it is provided by muscle cells (18), and this may account for the somewhat

**FIG 5.** The theory of enhanced sensitivity in control afforded by branch points in pathways. In this system there are three fluxes, $J$, $J_s$, and $J_x$ such that $J = (J_s + J_x)$. $X$ is a regulator of enzyme $E_2$, and, because there is no direct feedback from $E_2$ and $E_1$, $X$ changes $J_s$ at the expense of $J_x$, leaving $J$ unchanged.

required for oxidation, as could be the case in inactive muscle or in stress conditions in which fuel amounts are "primed" for a fight-or-flight response that, in modern civilization, is unlikely to occur, then the intracellular concentrations of fatty acid and fatty acyl CoA will increase, and these could cause damage. However, if triacylglycerol–fatty acid cycling occurs, the rate of esterification will be increased by raised concentrations of fatty acyl CoA, and triglyceride lipase activity will be decreased by the inhibitory effect of fatty acids; in this case the operation of the cycle would help to maintain relatively constant intracellular concentrations of fatty acid and fatty acyl CoA, thereby providing a buffering system. This is important because it is known that when concentrations of fatty acid, and especially fatty-acyl CoA, exceed the binding protein capacity, they are highly dangerous (2).

**Branch-point sensitivity and precision in control**

Much of metabolism is branched and although this may be necessary as an essential part of the diversity of reactions in the body, it is considered that branch points may also have been adapted for precision in regulation, as described below.

The increased sensitivity in control provided by the branch-point mechanism is similar in principle to that achieved in the substrate cycle: a continuous high flux in one branch of a pathway provides optimal conditions for the precise regulation of the (much smaller) flux in the other branch. Thus the increased sensitivity in the arm that has the smaller flux depends on the ratio of fluxes in the two branches. This is equivalent to the sensitivity improvement in cycling that is provided by the ratio of cycling rate to the overall flux (see above).

It can be shown that if a metabolic flux $J$ divides into two fluxes $J_x$ and $J_s$, and $J_s$ is regulated by factor $X$, the highest sensitivity of flux $J_x$ to changes in the regulator $X$ is achieved when $J_s \gg J_x$ (see Fig 5). Let us assume that $J_s$ represents a biosynthetic pathway for which $B$ is a precursor (eg. $J_s$ could represent the generation of purine and pyrimidine nucleotides for DNA and/or RNA synthesis and $B$, the precursor glutamine).

As $J_x$ is increased (by regulator $X$), the concentration of $B$ will tend to decrease and "deflect" flux from $J_x$ to $J_s$. However, assuming that enzyme $E_2$ is not saturated with $B$, this decreased concentration of $B$ will also reduce the flux through $E_2$ and, hence, will reduce $J_x$. Consequently, the increased $J_x$ brought about by regulator $X$ will be "opposed" by the decreased concentration of $B$, resulting in a less effective response of $J_s$ to $X$. This will decrease the precision of control of the flux through $J_x$ by $X$. If during the cell cycle, a precise increase in the rate of de novo pathways for purine and pyrimidine nucleotide synthesis

**FIG 6.** The oxidative and biosynthetic branches of the glutaminolytic and glycolytic pathways in lymphocytes and other rapidly dividing cells. Glutamine provides nitrogen for both the purine and pyrimidine rings: aspartate provides nitrogen and carbon for the pyrimidine ring. Glucose-6-phosphate provides both ribose and nicotinamide adenine dinucleotide phosphate (NADPH): via the pentose pathway, triose phosphate provides glycero-3-phosphate to phospholipids. Fluxes through glutaminolysis and glycolysis are massively in excess of the maximum capacities of the biosynthetic pathways. The breadth of the arrows indicates approximate relative rate (the broader the arrow, the faster the rate).
surprising response to injury: loss of body nitrogen and mobilization of fatty acids that is resistant to insulin.

The triglyceride–fatty acid cycle as an example of branch-point sensitivity

In the case of the triglyceride–fatty acid substrate cycle, it can be considered that a branch point occurs between the processes of esterification (cycling) and β-oxidation (Fig 7). Both brown adipose tissue and red skeletal muscle have a high capacity for fatty acid oxidation, but since this capacity will only be used infrequently, it is possible that cycling provides a branch point in the pathway that might enable the process of β-oxidation to retain maximum possible sensitivity to its regulator(s) in these tissues.

The high sensitivity will be achieved if the rates of lipolysis and esterification are high (that is, the cycling rate is high) and greater than the rate of β-oxidation. Under these conditions an increased rate of fatty acid oxidation to provide energy for the tissue will result in only a small decrease in the fatty acid concentrations because it is “protected” by the lipolytic process. The triglyceride–fatty acid substrate cycle can be seen as a dynamic buffer system since a small decrease in the concentration of fatty acid will not cause any marked opposition to the increased rate of oxidation (1, 3).

Postexercise oxygen consumption

Termination of exercise can occur almost instantaneously, but this is also accompanied by a time lag in the change in oxygen consumption. During this “recovery period,” more oxygen is consumed than is required to support the metabolism of the resting muscle. This extra oxygen consumption in recovery was noted in the early 1920s and for many years has been ascribed to the payment of an oxygen debt. More recently, it has been termed “post-exercise recovery oxygen” or “extra post-exercise oxygen consumption” (EPOC). The old name “debt” implied that it involved some form of reversal of the anaerobic processes that “saved” oxygen at the beginning of activity. However, this plays a minor role in explaining the magnitude of recovery oxygen after exercise.

Calculations have been carried out on the amount of oxygen that would be consumed by all of the processes considered to occur after exercise. These calculations indicate a large difference from the observed rate of oxygen consumption. Despite the obvious inaccuracies inherent in such calculations, the oxygen not accounted for by “classical” processes is large; some of this oxygen may be accounted for by stimulation of the rates of substrate cycles, after cessation of exercise, which would require energy and therefore oxygen. The significance of increased rates of cycling would be to increase the sensitivity of metabolic control so that the increased concentrations of fuels and increased rates of metabolism that occur during exercise can return gradually and smoothly to normal resting values during the recovery period and, in addition, that fuel reserves (eg, glycogen) can be restored to normal concentrations as quickly and effectively as possible but without causing hypoglycemia. Thus, maintenance of a somewhat elevated fatty acid concentration in the recovery period after exercise may provide an oxidative fuel for muscle and permit resynthesis of the important fuel store, muscle glycogen (19). There is now evidence that the rate of the triacylglycerol–fatty acid cycle is enhanced postexercise: it has been shown that at 3 h after cessation of exercise, 50% of the rate of the extra oxygen consumed can be accounted for by the rate of the triacylglycerol–fatty acid cycle (20). This suggests that this substrate cycle could play a major role in expenditure of energy after exercise and could therefore be of value in control of body weight.

Fatty acid oxidation as a branched pathway to provide for control in the immune system

The rate of conversion of [1-14C]oleate into 14CO2 by lymphocytes accounts for ≈1% of the total oleate utilized by the cells. However, addition of oleate (0.5 mmol/L) to isolated lymphocytes increases O2 consumption to an extent that suggests that the fatty acid contributes >30% to O2 consumption of the isolated lymphocytes, in comparison with that of glucose and glutamine (21). The apparent low rate of oxidation as indicated by conversion of [1-14C]oleate into 14CO2 may be due to the failure of acetyl CoA to be oxidized by the Krebs cycle. Thus, there is evidence that lymphocytes possess the specific enzymes for producing acetoaceteate (HMG-CoA synthase and lyase) (22) and that incubated lymphocytes do in fact produce ketone bodies (23). What is the role of ketogenesis in lymphocytes? It is possible that the primary role of this process is to maintain a high flux through a pathway in which acetyl CoA is an intermediate: such a high flux will act to “buffer” the concentration of acetyl CoA against any change in its rate of utilization in other pathways (Fig 8). Thus, during cell division, acetyl CoA may be required for several essential processes:

- synthesis of fatty acid and cholesterol for cell membranes,
- formation of malonyl CoA for chain extension of fatty acids for production of specific-chain-length fatty acids (eg, conversion of 18-C to 20-C or 22-C chains),
- use of acetyl CoA as an acetylation agent in covalent modification of regulatory proteins.

The ketogenic pathway would provide a further example of the mechanism of “branch-point sensitivity” in control of the rate of precursor utilization during the cell cycle. The buffering effect of the large flux into ketone bodies means that any increases in the rate of utilization of acetyl CoA in these pathways will not cause a major decrease in the concentration of acetyl CoA; this will mean that these processes can change their rate dramatically without suffering any limitation due to changes in acetyl CoA concentration. This role is consistent with the finding that fatty acids may be utilized and oxidized close to the maximal rate by lymphocytes and macrophages even in the fed state. This suggests
that fatty acids are taken up by the lymphocytes and macrophages not only to provide energy but to provide acetyl CoA for important functions in the cell that are related to immune function.

The possible role of polyunsaturated fatty acids as immunosuppressive agents

Background

More than one-half of the fatty acids in the human diet are unsaturated and, although desaturation can be achieved by enzymes in the liver, certain polyunsaturated fatty acids (PUFAs), which are required by the body, cannot be made in this way and must be provided in the diet; these are termed essential fatty acids. There are two major families of essential fatty acids, the n-6 family, derived from cis-linoleic acid, and the n-3 family, derived from α-linolenic acid. Linoleate and α-linolenate are transformed in the body to arachidonate and eicosapentanaoate, respectively. Both of these fatty acids are precursors for specific compounds that are known to play local messenger roles within and between cells: the prostaglandins, thromboxanes, prosta-cyclins, and leukotrienes.

It has been suggested that PUFAs have several roles in the health of an individual—and one of these roles is an effect on the immune system. In particular, it has been suggested that PUFAs possess immunosuppressive properties.

Major cellular components of the immune system include lymphocytes and macrophages. These cells are activated during an immune response: this activation is characterized by the synthesis and release of eicosanoids (eg, prostaglandins and leukotrienes) and cytokines (eg, interleukins) from macrophages. Immune processes are ongoing and in most individuals do not produce clinically detectable inflammation. However, an overactive immune system can result in autoimmune disease in which the host tissues are attacked. Such diseases include rheumatoid arthritis, in which the immune-activating agent is related to normal host tissue components, and systemic lupus, in which there are acquired abnormalities of the immune system, which contribute to the sustained nature of the inflammatory process. There is considerable evidence that inflammatory cytokines such as IL-1 and tumor-necrosis factor (TNF) play a major role in the aggressive activation and proliferation of lymphocytes and macrophages in chronic inflammatory diseases such as rheumatoid arthritis, which is the single biggest cause of disability and morbidity in the United Kingdom today. There is no cure for the disease, or indeed for autoimmune disease in general; in severe cases treatment has typically involved immunosuppressive drugs with unwanted and often dangerous side effects, or joint replacement, which inevitably places a burden on monetary resources in a health service. The influence of diet on the clinical manifestations of these diseases is highly controversial, and although clinical trials have attempted to determine whether PUFAs have beneficial effects on the severity and time of onset of several autoimmune diseases, the results from these trials have been inconclusive (24). It has become clear from these studies that a great deal remains to be learned about the biochemical functions of fatty acids in the immune system before their clinical utility can be satisfactorily assessed.

Lymphocyte proliferation and fatty acids

Proliferation of lymphocytes plays an important part in the response of the immune system to an immune challenge and, therefore, regulation of the rate of proliferation is central to regulation of the immune response. The initiation of the proliferative response of lymphocytes involves the plasma membrane in several different ways, all of which are considered to have a role in the control of proliferation: hydrolysis of phospholipids to yield arachidonic acid (a precursor of some eicosanoids); hydrolysis of phosphatidylinositol to generate the second messengers, diacylglycerol and inositol trisphosphate; increased ion permeability; increased substrate transport; increased activities of membrane-bound enzymes such as adenyl and guanyln cyclase, leading to increased concentrations of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP); secretion of interleukin-2 (IL-2); and expression of IL-2 and transferrin receptors. The functions of the plasma membrane are known to be influenced by the fatty acid composition of the membrane phospholipids, probably via effects on fluidity. In addition, because proliferation may be regulated, at least in part, by derivatives of long-chain polyunsaturated fatty acids, such as prostaglandins and diacylglycerol, it would not be surprising if changes in fatty acid composition of the cell membrane could influence proliferation. Is this the case?

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**FIG 8.** Branch point sensitivity for the ketogenic pathway in lymphocytes. Lymphocytes have a high rate of formation of acetoacetate from endogenous substrate that is presumed to be fatty acids. The role of this pathway in lymphocytes is considered to be the maintenance of a constant concentration of acetyl-CoA despite marked variations in its rate of utilization by these cells for synthesis of specific fatty acids for chain extension of fatty acids for cholesterol synthesis, and for acetylation of proteins (eg, histones). It may also allow extra ATP via the Krebs cycle at a specific time during the cell cycle.
A systematic investigation of the effects of a wide range of fatty acids on thymidine incorporation into rat-lymph-node lymphocytes stimulated by concanavalin-A (Con-A) was carried out (25). The effects of saturated (myristate, palmitate, and stearyl), monounsaturated (oleate), $\omega-6$ polyunsaturated (linoleate and arachidonate), and $\omega-3$ polyunsaturated (linolenate, eicosapentaenoate, and docosahexaenoate) fatty acids were studied. The concentrations of fatty acids used were physiological (30–500 $\mu$mol/L) and the fatty acids were added to the cell-culture medium as preformed complexes with bovine serum albumin. All the fatty acids tested had the ability to inhibit thymidine incorporation into lymph-node lymphocytes. However, the extent of inhibition depended on the particular fatty acid, the fatty acid concentration, the time during culture of fatty acid addition, and the duration of exposure of the cells to fatty acid. Generally, unsaturated fatty acids were more inhibitory than saturated fatty acids; the greatest inhibition of proliferation was caused by eicosapentaenoate and arachidonate, and the least inhibition was caused by myristate and palmitate.

These results confirm and extend the previous observations that fatty acids can inhibit in vitro lymphocyte proliferation stimulated by phytohaemagglutinin (PHA). Both these previous and the more recent studies (25) were performed with cell preparations containing a mixture of T and B lymphocytes. However, the specificity of Con-A and PHA as T-cell mitogens implies that the fatty acids exert their inhibitory effects on T-lymphocyte proliferation. Using the B-lymphocyte mitogen, lipopolysaccharide, Calder et al (26) found that fatty acids also inhibit B-lymphocyte proliferation; all fatty acids, however, caused less inhibition of B-cell proliferation than of T-cell proliferation, indicating that B lymphocytes are less susceptible to the inhibitory effects of fatty acids.

Lymphocyte proliferation is supported by the production of IL-2 by activated T cells. The appearance on the cell surface of receptors for IL-2 and transferrin is also required. It has been shown that unsaturated fatty acids inhibit the production of IL-2 by Con-A-stimulated lymphocytes isolated from rat lymph nodes and human peripheral blood (27). Fatty acids did not affect expression of the IL-2 receptor, but they suppressed transferrin receptor expression by up to 50% (27).

Macrophage functions and fatty acids

Macrophage adhesion is important in vivo interactions such as binding to endothelial cells and migration into tissues. Using a sensitive method to measure adhesion of macrophages to different surfaces. Calder et al (28) found that saturated fatty acid enrichment enhanced macrophage adhesion compared with enrichment in PUFA, which tended to decrease adhesion.

Phagocytosis is a major membrane-associated event in which particles are bound to membrane receptors and then are surrounded by the cell membrane, forming phagocytic vesicles, which are subsequently internalized and the contents are digested. Macrophages enriched with saturated fatty acids showed decreased rates of phagocytic uptake of unopsonized zymosan (boiled yeast cell walls) while those enriched with PUFA displayed increased phagocytic activity. This suggests that membrane fluidity is an important determinant of phagocytic activity; indeed, macrophage phagocytic activity was highly correlated with variables of membrane fluidity determined from the phospholipid fatty acid composition. Macrophages enriched with the fish-oil-derived fatty acids showed lower phagocytic activity than expected on the basis of the expected change in membrane fluidity of these cells (28).

Dietary fats and autoimmune diseases

Some time ago it was suggested that dietary fats, particularly plant-oil-derived $\omega-6$ PUFA's, may be immunosuppressive, and so could be of use in the treatment of diseases involving an overactive immune system. In recent years some evidence has been presented indicating that $\omega-3$ PUFA-rich fish oils may be particularly immunosuppressive, and so may be of use in protection against, and treatment of inflammatory or autoimmune diseases. However, the effects of fish oils on inflammatory and autoimmune disorders have not been extensively investigated. Much of the evidence in favor of these oils in the beneficial treatment of various disorders is anecdotal in nature, and in some instances it appears that $\omega-6$ PUFA-rich plant oils may be just as detrimental. Although dietary supplementation with $\omega-6$ or $\omega-3$ PUFA's has been successfully used in the treatment of some inflammatory diseases, several clinical trials of such diets have been unsuccessful in providing clear evidence of an improvement in the clinical condition of patients. Recently, it was suggested that "before further necessarily large studies of lipid supplementation are undertaken more needs to be understood about the effect of fatty acids before further clinical trials are undertaken" (29). Although these comments were made in reference to clinical trials involving multiple sclerosis patients, they apply equally well to other inflammatory disorders such as rheumatoid arthritis, systemic lupus erythematosus, and asthma and indeed to the general area of the effects of dietary fatty acids on cells of the immune system and their response to an immune challenge.

Plasma fatty acid, free tryptophan, and branched-chain amino acid concentrations and fatigue

Fatigue is defined physiologically as the inability to maintain power output. It is important for all of the population from the elite athlete to the patient suffering from injury, viral infection, surgery, or chronic fatigue syndrome. Much of the extensive research into factors causing fatigue has been done by physiologists and biochemists who are primarily interested in either nerves and nervous function or in the chemical and biophysical aspects of energy provision for the cross-bridge cycling in muscle. Another field of biochemistry, which has only recently been applied to exercise, is that of metabolic-control logic. This logic will be applied to our knowledge of amino acid and fatty acid metabolism to suggest a novel cause of fatigue. One important role of some amino acids is as precursors for certain brain neurotransmitters—in particular, the monoamines. One of these amino acids is tryptophan, which is converted in the brain to the neurotransmitter 5-hydroxytryptamine (5-HT).

There is some evidence to support the view that if the concentration of a neurotransmitter in the brain is decreased, this may limit the rate of neuronal firing in some parts of the brain, especially if the rate of neuronal firing is high. This inability of one part of the brain to function satisfactorily because of a decrease in neurotransmitter concentration can result in changes in behavior. For example, in some parts of the brain, a decrease in the concentration of the monoamine neurotransmitters (noradrenaline, dopamine, and/or 5-HT) can result in depression.
In addition, there is evidence to support the view that an increase in the level of 5-HT in the brain can result in tiredness and sleep. It is therefore suggested, as a working hypothesis, that an increase in the concentration of this neurotransmitter in certain areas of the brain might ensure a high rate of neuronal firing in a specific part of the brain, which then increases the sensitivity to fatigue. This would be called central fatigue. The logic underlying the hypothesis is as follows:

- Tryptophan is converted in the brain to a neurotransmitter known as 5-HT. This neurotransmitter may be involved in control of tiredness and sleep.
- Branched-chain amino acids (leucine, isoleucine, and valine) are not taken up by liver but by muscle, and their rate of uptake is increased during exercise. Both branched-chain amino acids and tryptophan enter the brain on the same amino acid carrier, so competition between the two types of amino acids for entry into brain can occur.
- An increased concentration of tryptophan in the brain will increase the rate of formation of 5-HT and hence will increase the concentration of this neurotransmitter in the brain. This could result in increased firing of some 5-HT neurones and this might result in central fatigue; that is, it might increase the mental effort necessary to maintain the pace of running or the level of activity of the exercise.
- Tryptophan is unique among amino acids in that it is bound to plasma albumin, so it exists in a bound form and a free form, which are in equilibrium; this equilibrium is changed in favor of free tryptophan when the plasma fatty acid concentration is raised above \( \approx 1 \text{ mmol/L} \). This change is probably caused by the binding of fatty acids to albumin.

The evidence to support these four points is presented elsewhere (30–33) (Fig 9).

In exercise, either intermittent or continuous, blood catecholamine concentrations are elevated, which results in fatty acid mobilization from adipose tissue, which results in an increase in the plasma fatty acid concentration. If there is precise control between the mobilization of fatty acids and the extent of vasodilation in muscle, the increased rate of fatty acid oxidation by muscle may not require much of an increase in the plasma concentration of fatty acids. Hence, the free tryptophan concentration may not change very much. However, if this coordination is poor—due to lack of training or due to hypoglycemia—the blood fatty acid concentration could be increased to sufficiently high concentrations to increase the plasma concentration of free tryptophan. Furthermore, in intermittent exercise, in which there is usually a greater dependence on "anaerobic" exercise and, therefore, less opportunity to oxidize these fatty acids, the plasma concentrations of fatty acid could rise above 1 mmol/L. An increase in the plasma fatty acid concentration plus a decrease in that of branched-chain amino acids could, thus, markedly influence the plasma concentration ratio of free tryptophan to branched-chain amino acids.

In prolonged exercise it seems likely that muscle will use branched-chain amino acids for energy when the muscle glycogen is depleted. Similarly, when the liver glycogen store is depleted, fatty acid mobilization may increase, raising the blood concentration of fatty acids above 1 mmol/L, so that the plasma concentration of free tryptophan will increase. Thus, an interesting possibility is that fatigue, which is caused by the depletion of glycogen in muscle, could be due not to a direct effect of glycogen depletion on the muscle, but to an increase in the concentration of 5-HT in a specific area of the brain. Failure of the motor center in the brain to stimulate muscle to contract would mean that the power output would have to fall, i.e. fatigue would be due to a change in the balance of the concentrations of key amino acids in the blood but initiated by depletion of liver and muscle glycogen stores!

Several lines of evidence support the above hypothesis. Athletes during various events have taken a solution containing branched-chain amino acids sufficient to maintain the resting value of the ratio of the concentrations of free tryptophan to branched-chain amino acids. For example, in a Stockholm marathon, 193 volunteers consumed a drink containing either a mixture of branched-chain amino acids or a placebo. For the slower runners (3.05–3.30 h to complete this marathon) performance was improved by taking branched-chain amino acids. The difference in time at this pace would mean an improvement in performance of 5–6 min (34).

A common anecdotal report from athletes who had taken branched-chain amino acids during a race was that they felt "better" mentally during the race and, in addition, that they were more mentally alert for some time after the race. This anecdotal information prompted a study into whether branched-chain amino acids taken during exercise can influence mental performance after cessation of exercise.

The Stroop color and word test (CWT) was given to 16 subjects who participated in a 30-km cross-country race. Research on the CWT has established that the test provides a useful tool in the study of neuropsychological and cognitive processes. In the group who took the branched-chain amino acids during the race, the performance in this test improved after the race compared with before the race, while no statistically significant difference was found for the subjects who took the placebo drink (35).

Similar findings have been found for soccer players, which suggests that effects of branched-chain amino acids may be important in many areas of physical activity.

Although much of the discussion has focused attention on fatigue in relation to performance of athletes, this should not conceal the potential clinical importance of these ideas. Fatigue is a factor that can decrease the rate of recovery and increase
the length of hospital stays after surgery, injury, and infection. And, of considerable importance, the cause of chronic fatigue syndrome is still unknown. The idea that plasma amino acid concentration changes may play a role in fatigue opens up a new line of investigation and suggests a new role for administration of solutions containing branched-chain amino acids.

References


Discussion

Martijn Katan: I was fascinated by your thoughts about the role of tryptophan in fatigue. Have you tried to give people extra tryptophan and see the effect on subjective feelings of fatigue?

Eric A Newsholme: No. We would wish to use 5-hydroxytryptamine receptor agonists and antagonists to test what they do to the performance of the rat on the treadmill. I believe there is such work being done in North America. That seems to me a better way of testing the hypothesis. More information should be obtained in relation to the neurophysiology.

Anton Wagenmakers: You didn’t do the experiment (tryptophan supplementation), but a Spanish group did. They observed a marked improvement of performance! I also would like to ask you another question. I heard you say several times that you appreciate now that the increase in fatty acid concentration is...
really a more important determinator of the branched-chain-amino acid/tryptophan ratio than is the decrease in branched-chain-amino acid concentration during exercise. My question then would be: Why don’t you use the normal carbohydrate supplements that all athletes use these days to prevent increases in fatty acid concentration? That will be a natural and safe way to influence the branched-chain-amino acid/tryptophan ratio. We understand most of exercise physiology, but not of the brain physiology of amino acids. Potentially, there are a lot of pitfalls, and sometimes we may even do dangerous things to athletes by providing them with amino acids.

Newsholme: About your first comment. I would not want to give the impression that this is the only cause of fatigue. If I have given this impression, then I want to correct it. There are many causes of fatigue. This is one of them, and, of course, as we have heard this morning, it is difficult in experiments on nutrition to make certain that you are changing one factor rather than several, which could also influence the system. Yes, I am sure that one of the reasons why glucose may be beneficial in performance is that not only is it providing the fuel, but it may also be providing an inhibitor of fatty acid mobilization, and to that extent may be lowering the fatty acid and free tryptophan concentrations. However, free tryptophan is not easy to measure; it does take time and effort, and I suspect that is why it has not been measured very often. We question how quickly the free tryptophan returns to normal. We wonder whether feeding glucose, particularly during the event itself, will have little effect on fatty acids because a degree of insensitivity to insulin occurs during exercise, probably due to the catecholamines. So, we would wish to reinvestigate the effects of glucose, branched-chain amino acids plus glucose, and branched-chain amino acids only to see whether it is possible to get any more definitive and useful information.

Wagenmakers: So you know that in Maastricht we also work on a different hypothesis on the effects of branched-chain amino acids. We certainly have shown in healthy individuals that when you provide branched-chain amino acids in amounts that are larger than you use, ammonia production increases during exercise. This is generally seen as a bad sign during exercise. Working in Liverpool with Richard Edwards, we gave branched-chain amino acids to patients with a glycogen-breakdown defect, McArdle disease, and there we saw a marked deterioration of performance during exercise, following that amino acid gift. There may be a message there that branched-chain amino acids not only affect central fatigue (potentially in the way you propose), but there also may be another effect in the muscle that in fact may overrule the central effect and lead to deterioration of exercise performance.

Newsholme: It wouldn’t surprise me if the mechanism of control is much more complicated than I have proposed. More importantly, there is the problem of providing the correct amount of branched-chain amino acids. To do the right thing at the right time, the prescription has to be accurate, and I think we are not quite clear about what is the precise prescription at this time.

Frits Muskiet: When you increase tryptophan in healthy subjects, you see an increase of 5-hydroxyindoleacetic acid in the urine. This is sometimes used as a proof that serotonin production is increased, but does this really mean that serotonin has been synthesized for purposes that have really been sensible? It doesn’t mean that serotonin as a neurotransmitter has really been produced.

Newsholme: I fully accept that. We have, of course, measured 5-HT in those specific parts of the brain. They do increase but I very much take your point that this does not prove it. We have to do much more “dissection” using the pharmacological approach.