Recent Developments in Metabolism That Impinge on Research Into the Nature and Treatment of Diabetes Mellitus

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It has been established that adenosine, its agonists, or antagonists can cause dramatic changes in insulin sensitivity in isolated soleus muscle and, moreover, can modify changes in sensitivity caused by pathophysiological conditions. Addition of adenosine deaminase to the incubation medium, which is known to lower the concentration of adenosine, increases the sensitivity of glycolysis to insulin. Addition of an adenosine-receptor agonist *decreases* sensitivity by about 10-fold, whereas addition of an adenosine-receptor antagonist *increases* sensitivity by about 10-fold. The latter totally removes the resistance of glucose utilization to insulin in the isolated soleus muscle obtained from either the genetically obese rat or from the rat fed a high sucrose diet. These findings strongly support the view that changes in insulin sensitivity in muscle can be brought about either by acute changes in the local concentration of adenosine or in the affinity or number of receptors for adenosine in muscle. However, in many of the conditions investigated, in which insulin sensitivity in muscle is changed, there was no correlation between the change in the adenosine content of the muscle and altered insulin sensitivity.

It has also been shown that prostaglandin E_1 can increase dramatically the sensitivity of glycolysis to insulin and that this is a specific effect of prostaglandins of the E series. It is not produced by prostacyclins, thromboxanes, or leukotrienes. It is unclear if there is a relationship between the effects of adenosine and prostaglandins.

Chronic elevation of catecholamines may increase the sensitivity of glucose utilization to insulin and also increase the rate of thermogenesis by substrate cycling. Thus, resistance to catecholamines in muscle could result in both insulin resistance and impaired thermogenesis. Recent work has emphasized the important role of glutamine in the immune system, yet little or no work has been done on glutamine metabolism in diabetes mellitus.

Despite an enormous research effort over the past 25 yr, the mechanism of insulin action is still unknown. This research effort has been supported by the pharmaceutical industry, charities, and government-supported bodies on the basis that biochemical knowledge of the mechanisms will permit the development of hypoglycemic agents to replace insulin. However, it can be argued that, particularly for treatment of type II diabetes mellitus, improving the sensitivity of specific tissues to endogenous insulin is likely to be a more fruitful field of investigation for the development of novel hypoglycemic agents. A number of studies have been done that suggest that some small molecules and some conditions influence dramatically insulin sensitivity, and these may offer the basis for the development of such agents.

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FACTORS THAT CAN MODIFY INSULIN SENSITIVITY OF MUSCLE AND THEIR POSSIBLE IMPORTANCE

Adenosine

Work in the author's laboratory has demonstrated that adenosine increases the sensitivity of the rate of glucose utilization and lipolysis to insulin in adipose tissue (1), but that it decreases the sensitivity of glucose utilization to insulin in skeletal muscle (2). Because muscle is considered to be the most important tissue for disposal of glucose in response to insulin, attention in recent years has focused on muscle and its sensitivity to insulin. Much of the work in the author's laboratory has been done with the isolated, incubated soleus muscle of the rat; the following important observations have been made: addition of adenosine deaminase to the incubation medium, which is known to lower the concentration of adenosine, increases dramatically the sensitivity of glucose utilization to insulin; in contrast, addition of an adenosine-receptor agonist (i.e., 2-chloroadenosine) to the incubation medium decreases about 10-fold the sensitivity of this process to insulin; addition of an adenosine-receptor antagonist (i.e., 8-phenyltheophylline) increases sensitivity by about 10-fold (3); and addition of this receptor antagonist not only influences insulin sensitivity of soleus muscle from a normal animal, but it also removes the resistance of glycolysis to insulin in the isolated soleus muscle ob-

tained from either the genetically obese rat (Zucker rat) or from the rat fed a high sucrose diet (4,5). Finally, metabolic symmetry is maintained, because addition of a receptor agonist (2-chloroadenosine) totally removes the increased insulin sensitivity of glycolysis to insulin in the soleus muscle obtained from the rats subjected to cold exposure for 2 days (6). This work suggests that specific adenosine receptors are involved in these effects; further studies are necessary to identify the type of adenosine receptor(s) that is present in skeletal muscle. If a novel adenosine receptor is present in skeletal muscle that proves to be unique to this tissue, this would provide an opportunity for medicinal chemists to provide specific and novel antagonists that could improve sensitivity of glucose utilization in muscle to insulin.

Prostaglandins

It has been found that prostaglandins of the E series (PGE_1 and PGE_2) can improve insulin sensitivity in the isolated incubated soleus muscle (7). Indomethacin, which inhibits prostaglandin formation, markedly decreases the sensitivity of glycolysis to insulin (7). Because PGE_2 is produced from arachidonic acid, these findings would suggest that diets high in polyunsaturated fatty acid could also improve insulin sensitivity. Of interest is the idea that conversion of linoleic acid, a common essential fatty acid in the diet, to arachidonate is severely limited in humans by the activity of Δ^6-desaturase and, therefore, this limitation can be overcome by supplementation of the diet with γ-linolenic acid. This, therefore, should allow, if the hypothesis is correct, a higher rate of synthesis of arachidonic acid. It is important to note that dietary supplementation with this fatty acid improves some of the complications of diabetes mellitus (8).

Catecholamines

The effect of an acute injection of catecholamines is to decrease the sensitivity of glucose utilization to insulin. This is probably caused by enhanced rates of glycogen breakdown and increased rates of mobilization of fatty acids (9). But what about chronic effects? Several physiological conditions in which the catecholamine level is raised are associated with an increase in the sensitivity of glucose metabolism to insulin (e.g., cold exposure and exercise training [10,11]). It has been shown that the soleus muscles removed from animals subjected to these conditions and incubated in vitro exhibit increased sensitivity of glucose utilization to insulin; it is suggested that a chronic increase in the level of catecholamines is involved in this improvement in insulin sensitivity. This suggestion is based on the fact that it has been shown that chronic treatment in vivo with adrenaline or β-adrenoceptor agonists increases the sensitivity of glucose utilization by muscle incubated in vitro to insulin (12,13).

Exercise

Endurance exercise increases the rate of utilization of all metabolic fuels. Thus, it decreases the glycogen content of both the liver and muscle. The latter may result in an increase in the activity of glycogen synthase and consequently may decrease the requirement for insulin in the stimulation of glycogen synthesis after a carbohydrate meal. In addition, it has been shown that, in normal subjects, endurance training markedly increases insulin sensitivity, so that very much lower concentrations of insulin are required to control the blood glucose concentration after an oral glucose load (14). For this reason, studies are being conducted to identify a possible exercise factor that might be responsible for this improvement in sensitivity. There is no doubt that a single bout of exercise produces a factor that increases the sensitivity of glucose utilization of skeletal muscle to insulin, but the evidence, in experimental animals, is that it is very short-lived (possibly only for a few hours) (15). However, exercise training increases the sensitivity of glucose utilization by isolated muscle to insulin for more than 48 h but probably less than 72 h (15). The mechanism underlying this longer term effect may be very important. It would be of interest if it was related to the chronic affect of catecholamines. Further work, regarding the identity of the factor responsible for the prolonged increase in insulin sensitivity after exercise training could provide information that might eventually lead to the development of novel hypoglycemic agents.

Insulin resistance and thermogenesis

Whatever the mechanism for the chronic effect of catecholamines described, the finding leads to an hypothesis for a common mechanism to explain both decreased rates of thermogenesis (not in the basal state, but in response to thermogenic stimuli) plus insulin resistance. Decreased rates of thermogenesis in response to thermogenic stimuli are common characteristics in some cases of obesity and type II diabetes mellitus.

There is considerable evidence to support the view that catecholamines can stimulate the rate of substrate cycles (see refs. 16–19 for concept and role of such cycles). Increased rates of cycling will not only increase sensitivity in metabolic control, but will also increase the rate of thermogenesis (20). It is suggested, therefore, that the increased rate of thermogenesis under conditions when catecholamines are elevated is caused, in part, by increased rates of substrate cycling. It is also suggested that catecholamines play an additional, but totally different role (i.e., they chronically increase the sensitivity of glucose utilization in muscle to insulin). This latter effect of catecholamines is not caused, as far as the author is aware, by an effect on substrate cycling, but may be achieved by changing the effectiveness of locally produced modulators. If these two quite separate effects of catecholamine are considered together, they could explain that, at least in some cases of obesity in experimental animals and in human, insulin

resistance coexists with decreased rates of thermogenesis in response to thermogenic stimuli (e.g., the rate of thermogenesis is decreased in response to glucose and/or in response to exercise) (21–23). Thus, if the muscle of these animals/subjects were insensitive to catecholamines, the rates of cycling would be lower, resulting in decreased rates of thermogenesis together with decreased insulin sensitivity of muscle, which would result in insulin resistance in vivo. A similar condition would result from a lower activity of the sympathetic nervous system, which has been reported in some obese subjects (24), or by pharmacological blockade of β-adrenoreceptor stimuli (25).

The author believes that this discussion provides prima facie evidence to support the hypothesis of a common mechanism for the control of thermogenesis, in response to specific stimuli, and the control of the sensitivity of glucose utilization in muscle to insulin. Because catecholamines are known to stimulate the rates of substrate cycling, which will increase the rate of conversion of chemical energy into heat, an elevation in the plasma and tissue catecholamine concentration, either acutely or chronically, will increase the rate of thermogenesis: but chronic elevation in the catecholamine levels increases the sensitivity of glucose utilization by muscle to insulin. Consequently, lowering the concentration of catecholamines in the plasma or within the muscle will result in both decreased thermogenesis and decreased sensitivity to insulin. It is of interest therefore that some pharmaceutical firms are interested in novel β-adrenoreceptor agonists as antiobesity plus hypoglycemic drugs (26,27).

GLUTAMINE: THE IMMUNE SYSTEM AND DIABETES MELLITUS

— Another recent metabolic discovery, which has recently developed into an important clinical nutritional area, is the role of glutamine for cells of the immune system and those involved in recovery and repair (28,29). So far, these findings have not impinged on the diabetic area of research—perhaps they should!

Systematic studies in the 1970s on the maximum activities of key enzymes of carbohydrate and fat metabolism in muscle provided information on the types of fuels utilized by different muscles and their maximum contribution to ATP formation to support contractile activity (30). This enabled a systematic and comprehensive analysis to be made of the fuels used by different muscles from different animals across the animal kingdom (31). More recently, a similar approach to the study of fuels used by lymphocytes and macrophages has been conducted and has provided, for the first time, evidence that these cells can use glutamine for energy formation and, indeed, that this fuel could be quantitatively more important than glucose (28,29,32). Until this work, it had been considered that glucose was the major, if not the only, fuel to be used by lymphocytes. Detailed quantitative studies not only on the enzymology, but also on the fate of glutamine and isotopic experiments, have shown that glutamine is only partially oxidized by this cell. Much of it is converted into glutamate, aspartate, and lactate rather than completely oxidized to CO_2. This partial oxidation has been termed glutaminolysis.

The role of the partial oxidation of glutamine (glutaminolysis) in rapidly dividing cells has been considered to be the provision of both nitrogen and carbon for precursors for synthesis of macromolecules (e.g., purines and pyrimidines for DNA and RNA) and also for energy formation (33). However, there are problems with both explanations. Quantitative information, provided by the work of the author with lymphocytes, shows that the rate of glutaminolysis is markedly in excess (>400-fold) of that of the precursor requirements (34). And, if energy generation per se was the major reason for high rates of glutamine utilization, it would be expected that more of the carbon skeleton of glutamine would be converted to acetyl-coenzyme A for complete oxidation by the Krebs cycle (i.e., that glutamine oxidation rather than glutaminolysis would occur). Further, a high proportion of the energy for resting lymphocytes and macrophages can be obtained by the oxidation of fatty acids. So, why is glutamine used at such a high rate?

It may have a more important role than provision of energy. On the basis of new quantitative approaches to metabolic control (35), the author has suggested that the high rates of both glutaminolysis and glycolysis provide ideal conditions for the precise and sensitive control of the rate of use of the intermediates of these pathways for biosynthesis precisely at the time required by the biosynthetic process during the cell cycle (e.g., glucose-6-phosphate for ribose-5-phosphate formation; glycerol-3-phosphate for phospholipid formation; glutamine for purine and pyrimidine syntheses; and aspartate for pyrimidine synthesis). High rates of glycolysis and glutaminolysis can thus be seen as part of a mechanism of control to permit synthesis of macromolecules when required without any need for complex regulatory mechanism or extracellular signals to make more glucose or glutamine available for the rapidly dividing cells. This suggests that a high rate of glutamine utilization is essential for growth and cell division and, therefore, is a fundamentally important process in the immune system. Consequently, any decrease in the flux through this glutamine pathway—even if small—could impair the functioning of the immune system. In support of this, it has been shown that a decrease in the extracellular level of glutamine can markedly decrease the rate of proliferation of lymphocytes in culture. If this occurs in vivo, it would decrease the effectiveness of this defense mechanism. There is now evidence that, in some conditions in which immunosuppression is known to occur (e.g., burns), the plasma glutamine level is markedly decreased (36).

It might be considered that the glutamine is made available for the cells of the immune system from dietary protein by digestion, but this is not the case. Most, if not all, of the glutamine absorbed into the intestinal cells is metabolized by these cells. Thus, the blood glutamine level is maintained by tissues that can synthesize glutamine, mainly liver and muscle, and it seems that skeletal muscle plays the dominant role (37). In other words, skeletal muscle can be considered to be part of the immune system.

The link between muscle and the immune system is plasma glutamine, just as plasma glucose acts as the link between liver and brain. Just as failure of the liver to produce enough glucose can cause malfunctioning of the brain (and even hypoglycemic coma), so malfunctioning of this aspect of muscle metabolism may impair the immune system (38).

If glutamine is an important immunostimulator and fuel for the immune system and if it is released from muscle in a controlled manner in relation to demand on the immune system, what happens in diabetes mellitus? Could this be another area where metabolic control is impaired? Knowledge of a change in the plasma glutamine level in varying types of diabetes mellitus could be extremely important. If it is decreased, then administration of glutamine or glutamine-containing peptides could be of immense importance in improving the functioning of some cells, including cells of the immune system. It is also possible to speculate that some of the changes observed in the long-term complication of diabetes might be caused, in part, by failure to maintain the plasma glutamine concentration at normal levels.

References

1. Green A, Newsholme EA: Sensitivity of glucose uptake and lipolysis of white adipocytes of the rat to insulin and effects of some metabolites. *Biochem J* 180:365–70, 1979
2. Espinal J, Challiss RAJ, Newsholme EA: Effects of adenosine deaminase and an adenosine analogue on insulin sensitivity in soleus muscle of the rat. *FEBS Lett* 158:103–106, 1983
3. Budohoski L, Challiss RAJ, McManus B, Newsholme EA: Effect of analogues of adenosine and methyl xanthines on insulin sensitivity in soleus muscle of the rat. *FEBS Lett* 167:1–4, 1984
4. Challiss RAJ, Budohoski L, McManus B, Newsholme EA: Effects of an adenosine-receptor antagonist on insulin-resistance in soleus muscle from obese Zucker rats. *Biochem J* 221:915–17, 1985
5. Budohoski L, Challiss RAJ, Cooney GJ, McManus B, Newsholme EA: Reversal of dietary-induced resistance in muscle of the rat by adenosine deaminase and an adenosine-receptor antagonist. *Biochem J* 224:327–30, 1985
6. Budohoski L, Challiss RAJ, Lozeman F, McManus B, Newsholme EA: Increased insulin sensitivity in soleus muscle from cold-exposed rats: reversal by an adenosine-receptor agonist. *FEBS Lett* 175:402–406, 1984
7. Leighton B, Budohoski L, Lozeman FJ, Challiss RAJ, Newsholme EA: The effect of prostaglandins E_1, E_2 and F_2 and indomethacin on the sensitivity of glycolysis and glycogen synthesis to insulin in stripped soleus muscles of the rat. *Biochem J* 227:337–40, 1985
8. Jamal GA: Prevention and treatment of diabetic distal polyneuropathy by the supplementation of γ-linolenic acid. In *Omega-6-Essential Fatty Acids* Horrobin DF, Ed. New York, Wiley-Liss, 1990, p. 485–504.
9. Challiss RAJ, Lozeman FJ, Leighton B, Newsholme EA: Effects of the β-adrenoreceptor agonist isoprenaline on insulin sensitivity in soleus muscle of the rat. *Biochem J* 233:377–81, 1986
10. Maron MB, Horvath SM, Wilkerson JE: Blood biochemical alterations during recovery from competitive marathon running. *Eur J Appl Physiol* 36:231–35, 1977
11. Leduc J: Catecholamine production and release in exposure and acclimation to cold. *Acta Physiol Scand* 53 (Suppl. 183): 1–44, 1961
12. Challiss RAJ, Budohoski L, Newsholme EA, Sennitt MV, Cawthorne MA: Effect of a novel thermogenic β-adrenoceptor agonist (BRL 26830) on insulin resistance in soleus muscle from obese Zucker rats. *Biochem Biophys Res Commun* 128:928–35, 1985
13. Budohoski L, Challiss RAJ, Dubaniewicz A, Kaciuba-Uscilko H, Leighton B, Lozeman FJ, Nazar K, Newsholme EA, Porta S: *Biochem J* 244:655–60, 1987
14. Bjorntorp P, Fahten M, Grimb G: Carbohydrate and lipid metabolism in middle-aged physically well trained men. *Metabolism* 21:1037–44, 1977
15. Langfort J, Budohoski L, Newsholme EA: Effect of various types of acute exercise and exercise training on the insulin sensitivity of rat soleus muscle measured in vitro. *Pflugers Arch* 412:101–105, 1988
16. Newsholme EA, Challiss RAJ, Crabtree B: Substrates cycles; their role in improving sensitivity in metabolic control. *Trends Biochem Sci* 9:27–32, 1984
17. Challiss RAJ, Arch JRS, Newsholme EA: The rate of substrate cycling between fructose 6-phosphate and fructose 1,6-bisphosphate in skeletal muscle. *Biochem J* 221:153–61, 1984
18. Challiss RAJ, Arch JRS, Crabtree B, Newsholme EA: Measurement of the rate of substrate cycling between fructose 6-phosphate and fructose 1,6-bisphosphate in skeletal muscle by using a single-isotope technique. *Biochem J* 223: 849–853, 1984
19. Challiss RAJ, Arch JRS, Newsholme EA: The rate of substrate cycling between fructose 6-phosphate and fructose 1,6-bisphosphate in skeletal muscle from cold exposed, hyperthyroid or acutely exercised animals. *Biochem J* 31:217–20, 1985
20. Newsholme EA, Challiss RAJ, Leighton B, Lozeman FJ, Budohoski L: A possible common mechanism for defective thermogenesis and insulin resistance in obesity. *Nutrition* 3:195–200, 1987
21. Ravussin E, Cheson KJ, Vernet O: Evidence that insulin, resistance is responsible for the decreased thermic effect of glucose in human obesity (Abstract). *J Clin Invest* 76:1268, 1985

22. Katzeff HL, O'Connell M, Horton ES: Metabolic studies in human obesity during undernutrition and overnutrion: thermogenic and hormonal responses to norepinephrine (Abstract). *Metabolism* 35:166, 1986
23. Zahorska-Markiewicz B: Thermic effect of food and exercise in obesity (Abstract). *Eur J Appl Physiol* 44:231, 1980
24. James WPT: Adrenergic mechanisms in obesity (Abstract). *Clin Physiol* (Suppl. 1):60–65, 1981
25. Leibel RL, Hirsch J: A radioisotopic technique for analysis of free fatty acid re-esterification in human adipose tissue (Abstract). *Am J Physiol* 248:E140, 1985
26. Smith SA, Sennitt MV, Cawthorne MA: BRL 35135: an orally active antihyperglycaemic agent with weight reducing effects. *New Antidiabetic Drugs*. Bailey CJ, Flatt PR, Eds. Smith-Gordon, 1990, p. 177–81
27. Mitchell TH, Ellis RDM, Smith SA, Robb G, Cawthorne MA: Effects of BRL 35135, a β-adrenoceptor agonist with novel selectivity, on glucose tolerance and insulin sensitivity in obese subjects. *Int J Obesity* 13:757–66, 1989
28. Ardawi MSM, Newsholme EA: Metabolism in lymphocytes and its importance to the immune response. *Essays Biochem* 21:1–44, 1985
29. Newsholme EA, Newsholme P, Curi R, Challoner MA, Ardawi MSM: A role for muscle in the immune system and its importance in surgery, trauma, sepsis and burns.
30. Newsholme EA, Leech AR: *Biochemistry for the Medical Sciences*. Chichester, England, John Wiley & Sons
31. Newsholme EA, Crabtree B, Newsholme P: Use of enzyme activities as indices of maximum rates of fuel utilisation. *Ciba Found Symp* 73:245–58, 1980
32. Newsholme P, Newsholme EA: Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochem J* 261:211–18, 1989
33. McKeehan WL: Glycolysis, glutaminolysis and cell proliferation. *Cell Biol Int Rep* 6:635–50, 1982
34. Szondy Z, Newsholme EA: The effect of glutamine concentration on the activity of carbamoyl-phosphate synthase II and on the incorporation of [^{3}H]-thymidine into DNA in rat mesenteric lymphocytes stimulated by phytohaemagglutinin. *Biochem J* 261:979–83, 1989
35. Crabtree B, Newsholme EA: A quantitative approach to metabolic control. *Curr Top Cell Regul* 25:21–76, 1979
36. Parry-Billings M, Evans J, Calder PC, Newsholme EA: Does glutamine contribute to immunosuppression after major burns? *Lancet* 336:523–25, 1990
37. Parry-Billings M, Leighton B, Dimitriadis GD, De Vasconcelos PRL, Newsholme EA: Skeletal muscle glutamine metabolism during sepsis. *Int J Biochem* 21:419–23, 1989