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EFFECT OF ALTERED NUTRITIONAL STATES ON INSULIN RECEPTORS[1]

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CONTENTS

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INTRODUCTION

In this review of the insulin receptor in states of altered nutrition, we primarily discuss obesity and its effect on insulin receptors and insulin action, as well as the effects of short- and long-term dietary restrictions and alterations. This is prefaced by a brief survey of the insulin receptor.

Review of the Insulin Receptor

The insulin receptor is a heterotetramer consisting of two extracellular alpha subunits, each 135,000 daltons, that are joined by disulfide bonds to two beta subunits, each 95,000 daltons, that span the plasma membrane. Insulin binding to the alpha subunit activates the cytoplasmic domain of the beta subunit, which in turn initiates a number of processes, including phosphorylation of tyrosine residues of the beta subunit of the receptor and tyrosine residues of exogenous substrates (51, 83) and activation of soluble intracellular ("second") messengers (17). It is still unclear how these early events relate to one another or produce the final biological actions of insulin such as enhanced glucose transport and utilization and synthesis of glycogen, lipid, and protein. Insulin receptors are affected by a number of biological agents, including chronic levels of circulating insulin and other hormones (26, 28, 36, 66); ionic milieu (9, 58), especially pH; metabolites, (37, 42); and the nature of the membrane lipids (39). Therefore, the biochemical or molecular bases for alterations in the insulin receptor in response to altered nutritional states may lie at least partially in one or more of these regulatory mechanisms.

The dose-response curves for the late biological effects of insulin are commonly more sensitive to insulin than are the curves for binding of insulin to its receptor or the effect of insulin on early postbinding events (e.g. tyrosine phosphorylation). This suggests that not all of the receptors need to be occupied for maximal biological responses to occur. This spare receptor concept predicts that a reduction in the number of insulin receptors (like a reduction of receptor affinity) is associated with a maximal insulin effectiveness that is the same though with reduced sensitivity (depicted as a rightward shift of the dose-response curve). Further, a reduction of maximal insulin responsiveness is associated with either a very severe receptor deficiency or more commonly a postbinding defect (47).

It should also be noted that the effect of insulin on a target cell is a function of the plasma insulin concentration and the target receptor concentration. Plasma insulin concentration is in turn a function of its rate of secretion from the pancreas and its rate of clearance from the plasma. Interactions between insulin and insulin receptors can in turn be amplified during signal transduction as a result of postreceptor events. We define postreceptor as any event in

insulin action that occurs distal to the α-subunit binding reaction and β-subunit autophosphorylation reaction.

The insulin-like growth factor type I (IGF-I) receptor is very similar to the insulin receptor. Insulin binds to the IGF-I receptor, albeit with an affinity that is 1–2 orders of magnitude lower than for insulin binding to the insulin receptor (68). Some of insulin's effects in certain tissues may be mediated via the IGF-I receptor, especially when the concentrations of insulin or of the IGF-I receptor are especially high.

A major regulator of the insulin receptor (affinity, number, subcellular distribution) and of target cell sensitivity to insulin at postreceptor sites is insulin itself. Typically, elevations of ambient insulin concentrations produce an immediate reduction in the affinity of the receptor for insulin (26), while chronically elevated levels of insulin lead in a few hours to a reduced number of receptors on the surface of cells and altered sensitivity at other sites (36).

MURINE MODELS OF INHERITED OBESITY

ob/ob Mouse

The ob/ob mouse is the model used most extensively to study the obesity syndrome and is the most hyperinsulinemic. The obesity is an autosomal recessive trait; 25% of the offspring are obese of genotype ob/ob, and 75% are lean with genotypes +/+ (25%) and ob/+ (50%) (76). Thus far the lean littermates of both genotypes are phenotypically indistinguishable (73). The ob/ob mice are hyperphagic, hyperglycemic, and hyperinsulinemic, and their chronic rate of tissue lipogenesis is markedly elevated over that of their lean littermates (76). In the fed state, insulin levels in the ob/ob mice are 10–100-fold higher than thin littermates, whereas plasma glucose levels are elevated by 2–4-fold (35, 48, 73). This difference suggests insulin resistance, which has been further documented in vitro and in vivo (76). By comparing insulin receptor binding studies and insulin action in obese vs thin littermates, we can define the nature of insulin resistance in ob/ob mice in various tissues. This requires assessment of the receptor-related defect as well as postbinding defects.

FED STATE

Liver The number of insulin receptors on isolated hepatocytes and liver membranes from ob/ob mice is only 20–30% of that in their lean littermates (32, 48, 73, 74). The decrease in number affects both the surface receptors and the total cellular insulin receptors (48). The residual receptors are functionally and structurally indistinguishable from normal (74). The decrease in number of insulin receptors is probably not due to the ob gene, as thin littermates heterozygous for the ob gene (ob/+) have the same number of

insulin receptors as homozygous thin mice (+/+) do (73). Further, the reduced number of receptors may be substantially improved by amelioration of the hyperinsulinemia (see below). The liver is also poorly responsive to insulin (e.g. in stimulating lipogenesis) presumably as a result of postbinding defects as well (56).

Muscle Heart and skeletal muscle preparations from ob/ob mice show markedly reduced ^{125}I-insulin binding (32, 54). Furthermore, the insulin dose-response curve for 2-deoxyglucose uptake is shifted to the right (54). This effect is due to a reduced receptor number, without any major alteration in affinity. Insulin resistance of skeletal muscle in the ob/ob mouse is also associated with postreceptor defects (54), because the response of 2-deoxyglucose uptake and total glucose utilization to a maximally stimulating insulin dose is markedly reduced, although according to one report there is no alteration in insulin receptor tyrosine kinase activity (82).

Fat As with liver and muscle, plasma membranes from epididymal (white) adipocytes have reduced numbers of insulin receptors (35). There is also a postreceptor defect; insulin very poorly stimulates glucose incorporation into total adipose lipids or triglyceride fatty acids in the fed ob/ob mice, while producing a 3–6-fold stimulation in adipose tissue of fed, thin littermates (56).

Specific insulin binding is reduced by 70% in wheat-germ purified preparations from brown adipose tissue of ob/ob mice (79). The reduced number of brown adipose insulin receptors, and the resultant reduction in receptor tyrosine kinase activity (relative to lean littermates) has been postulated as a contributing factor in abnormalities in brown adipose tissue function in ob/ob mice.

Lymphocytes The number of insulin receptors is reduced on thymocytes from ob/ob mice (72), without an alteration in receptor affinity. Since insulin at a high concentration fails to stimulate amino isobutyric acid transport into the thymocytes of fed ob/ob mice, while being effective in cells from lean littermates, we conclude that postbinding defects (in addition to receptor defects) are present.

Brain In contrast to other tissues, neither the number nor the affinity of insulin receptors on brain membranes from ob/ob mice are altered relative to those of lean littermates despite the reduction in liver membrane insulin receptor number (45). This result is also observed in different brain regions, including olfactory bulb, diencephalon, telencephalon, brain stem, and cerebellum.

MANIPULATIONS OF THE ob/ob MOUSE

Food restriction Pair-feeding studies have been performed in which obese mice are restricted to the same daily caloric intake as their thin littermates. Generally the body weight of ob/ob mice matches that of their thin littermates at the conclusion of the study (35, 73). Despite a normalized body weight, the percentage of body fat remains elevated, and although the glycemia is essentially normalized, the pair-fed ob/ob mice remain hyperinsulinemic relative to the thin littermates. The degree of hyperinsulinemia is, however, reduced by ~20-fold compared to fed ob/ob mice. In spite of the reduced hyperinsulinemia, adipocyte insulin receptor numbers are not improved (35) while the number of insulin receptors on liver plasma membranes is partially restored to levels intermediate between those of thin littermates and fed obese (73). Pair-feeding restores insulin responsiveness and sensitivity in skeletal muscle to a greater degree than it does in adipose tissue (2).

Acute fasting Fasting for periods of 24–40 hr lowers plasma insulin and glucose levels and results in a concomitant elevation in the number of insulin receptors on liver and heart membranes (32, 73). Despite these improvements in receptor number, acute fasting does not restore responsivity to insulin-stimulated glucose metabolism in the obese mice, which suggests that postbinding defects remain (2).

Chronic fasting A 12-day fast renders ob/ob mice hypoglycemic and completely eliminates their hyperinsulinemia (56). Concomitant with these changes, insulin receptor numbers are restored completely or almost completely in liver, fat, and muscle. However, liver and adipose tissue remain unresponsive to insulin stimulation of lipogenesis. In contrast, in muscle, sensitivity and responsivity of glucose metabolism to submaximal and maximally stimulating insulin levels are markedly enhanced compared to fed ob/ob mice (56).

Streptozotocin administration Streptozotocin lowers plasma insulin levels (presumably by destroying the insulin secreting cells) from ~30 ng/ml in the untreated ob/ob mice to ~2 ng/ml (56). Plasma glucose levels are double those seen in untreated ob/ob mice. Streptozotocin treatment restores liver insulin receptor numbers in proportion to its effect on plasma insulin, although the number is not completely restored to that seen in thin littermates. Similar to the results of the 12-day fast, insulin fails to stimulate lipogenesis in liver or adipose tissue of streptozotocin-treated ob/ob mice despite the elevation in receptor number (56).

SUMMARY Peripheral tissues of the genetically obese hyperglycemic mouse exhibit reduced numbers of functionally normal insulin receptors, probably as a consequence of their hyperinsulinemia. While this receptor loss probably contributes to impaired insulin action, various manipulations that reduce hyperinsulinemia and concomitantly restore insulin receptor numbers do not improve insulin action in the liver or fat tissue. Muscle tissue sensitivity and responsivity to insulin are restored when receptor number is restored, but developmental studies indicate that, even in this key tissue, impairment in the stimulation of glucose uptake and metabolism by insulin can be observed in the absence of reduced receptor number (41a). It is thus fair to conclude that postbinding defects in insulin action, still largely undefined, may play a greater role than does the deficiency of insulin receptor binding sites in the insulin resistance of the ob/ob mouse.

The db/db Mouse

In the genetically diabetic mouse (db/db), like the ob/ob mouse, inheritance is that of an autosomal recessive trait (76), and the syndrome includes hyperinsulinemia, hyperglycemia, and insulin resistance. It differs from the ob/ob mouse in that it develops ketoacidosis and consequently has a shorter life. Lipogenesis is chronically elevated in the young db/db mouse but declines as the mouse ages, while the abnormally high rate of gluconeogenesis continues unabated. Data are not as extensive in the db/db mouse as in the ob/ob mouse.

FED STATE

Liver Liver membranes from fed, 12-week-old db/db mice possess only 30 to 50% the number of insulin receptors of their thin littermates (73). Inclusion of 0.001% estrone in the diet of db/db mice (9 weeks old) eliminates their hyperinsulinemia and hyperglycemia, without affecting the hyperphagia and obesity. The estrone treatment, however, does not result in a significant restoration of insulin binding to liver membranes (67). No simple interpretation of the results of estrone treatment has emerged.

Muscle Insulin binding to skeletal muscle (plasma membrane preparations) from 12–20-week-old db/db mice is ~60% of the level of the lean mice (70). However, this same study indicates that younger db/db mice (4–5 weeks old) that are not as hyperinsulinemic as the 12-week-old mice do not exhibit diminished skeletal muscle insulin binding. However, insulin receptor autophosphorylation is reduced in both younger and older mice by 50 to 60% of that seen in lean mice. Since glucose transport by the perfused hind-limb of the younger db/db mice shows a lowered response to maximally stimulating insulin levels compared to thin littermates (16), postbinding defects probably account for all of the insulin resistance in younger db/db mice (and at least

some of the resistance in the older mice) and this defect may lie in the receptor autophosphorylation (70). The same report that indicated that skeletal muscle insulin receptor tyrosine kinase was unaltered in ob/ob mice, however, also reported no alteration in insulin receptor binding or kinase activity in skeletal muscle of 24-week-old db/db mice, and concluded that the insulin resistance of skeletal muscle of both of these obese rodent models could not be explained by reduced receptor kinase activity (82).

SUMMARY The db/db mouse basically behaves like the ob/ob mouse in that the hyperinsulinemia down-regulates insulin receptors. The two models of obesity may, however, exhibit some differences in the age of onset of receptor down-regulation and in the coupling of receptor binding to the receptor's tyrosine kinase.

New Zealand Obese

The New Zealand Obese (NZO) mouse inherits obesity in a polygenic manner, is hyperinsulinemic, hyperglycemic, and insulin resistant, and its insulin receptor numbers are reduced on liver membranes with no alteration in affinity (6). Curiously, implantation of islets from normal mice reduces the plasma glucose and insulin levels of the NZO mouse and restores insulin receptor numbers. Removal of the implanted islets restores the hyperinsulinemia and deficiency of insulin receptors (6). Thus in this model of obesity, the insulin resistance may in part be related to some pancreatic abnormality. The possible role of circulating antibody to the insulin receptor, detected in the NZO mouse by one group, is as yet undefined (44).

OBESITY IN MICE INDUCED BY GOLD THIOGLUCOSE

The intraperitoneal injection of gold thioglucose (GTG) into mice, presumably by damaging hypothalamic satiety centers, often results in obesity, typically associated with hyperinsulinemia by 7–8 weeks of age (i.e. younger mice) as well as an increased proportion of body fat (55). However, GTG mice do not demonstrate severe insulin resistance until the age of 16–20 weeks (i.e. older mice) when they are also hyperglycemic and hyperinsulinemic.

Fed State

LIVER Liver membranes from older GTG-obese mice have only 50–70% the number of insulin receptors as their thin littermates (73) without any alterations in receptor affinity. In both the younger and older GTG-obese mice, insulin fails to stimulate hepatic lipogenesis despite the less marked receptor reduction in the younger animals (55), which suggests possible postreceptor defects in insulin action.

MUSCLE As with liver, soleus muscle of older GTG-obese mice has fewer insulin receptors than muscle of thin littermates (54), associated with normal receptor affinity and a rightward shift in the insulin dose-response curve for uptake of 2-deoxyglucose. Like liver, the diminution in muscle receptor number (~30% decrease) is less marked than it is with the ob/ob mouse. The maximum rates of insulin-stimulated 2-deoxyglucose uptake, glycolysis, and glycogen synthesis are reduced in the older mice, which suggest an important role for postbinding defects, including a reduced insulin receptor tyrosine kinase activity in excess of reductions in insulin receptor number (53). However, others have found decreased sensitivity to insulin only in proportion to the receptor deficiency (20). This apparent discrepancy may be related to the duration of the acquired obesity.

FAT Glucose utilization by epididymal (white) adipocytes from younger GTG-obese mice is greater basally and in response to insulin than tissue from lean controls (20). The sensitivity to insulin is also enhanced, which may mean that white adipose tissue in younger GTG-obese is hyperresponsive to insulin. Adipose tissue from the older GTG-obese mice, however, is insensitive and unresponsive to insulin stimulation (55).

Brown adipose cells have a marked (60–70%) reduction in the number of insulin receptors, exceeding the reduction in receptor number found in skeletal muscle and liver (79), but the affinity of the receptors is unaltered, and in contrast with soleus muscle, tyrosine kinase activity per insulin receptor of brown adipose tissue is unaltered. Cooney et al (20), in their survey of glucose utilization by various tissues in the GTG-obese mouse, found brown adipose tissue to have the most reduced maximally insulin-stimulated glucose metabolism (relative to lean controls) and the most reduced insulin sensitivity. Thus, binding as well as postbinding defects exist for insulin action in brown adipose tissue of GTG-obese mice but the mechanism of the defect is incompletely defined.

CALORIC RESTRICTION Pair-feeding studies are used to restrict GTG mice to the same daily food intake as their thin littermates (73). Plasma glucose levels are indistinguishable from those of thin littermates as are the plasma insulin levels. Liver insulin receptors are almost totally restored under these conditions.

A 40-hour fast in GTG-obese mice causes a slight decrease in the body weight of GTG-obese mice, as well as a fall in plasma glucose and plasma insulin levels to below the levels seen in fed, thin littermates (54). Under such conditions, the number of insulin receptors on soleus muscle is almost completely restored to that seen in thin littermates, and furthermore, sensitivity and responsivity of glucose metabolism to insulin are restored completely to normal.

Summary

Overall, GTG-obese mice, like ob/ob mice, appear to possess fewer insulin receptors and the reduced number has an inverse relationship to the hyperinsulinemia. The reduction is not as great as that for the ob/ob mice, probably because of the less pronounced hyperinsulinemia. Like the ob/ob mouse, many insulin-stimulated metabolic processes in the GTG-obese mice are defective only partly because of the diminished insulin receptors. Importantly, muscle responds to a fast by totally normalizing its insulin receptor number and insulin action.

RAT MODELS OF INHERITED OBESITY

Zucker Fatty Rat

The inheritance of obesity in the Zucker fatty rat is as an autosomal recessive trait (11). Additionally, Zucker fatty rats are hyperphagic, resistant to insulin, hyperinsulinemic, and both their adipose tissue and their livers demonstrate increased lipogenesis (11). In contrast to some of the genetic syndromes in the mouse, they remain normoglycemic. The hyperinsulinemia is characteristic and progressive from four weeks of age, when plasma levels are 3–4-fold greater than normal, to twenty weeks, when it reaches 20-fold above normal and then falls to about 4-fold above normal at fifty-two weeks (18).

FED STATE

Liver Despite elevated circulating plasma insulin levels, young rats ($\leq$8 weeks) may have a normal number of receptors (18, 19). By 10–20 weeks of age, receptor numbers are decreased by 70% or more, coincident with plasma insulin levels that are up to 30-fold higher than controls (18, 49; J. Shemer, A. Ota, D. LeRoith, submitted for publication), and hepatic unresponsiveness to insulin even up to 400 ng/ml (80).

In 10–12-week-old fatty rats, insulin fails to suppress hepatic glucose production (80). At 16 weeks, insulin receptor tyrosine kinase activity is elevated in livers from fatty rats (J. Shemer, A. Ota, D. LeRoith, submitted for publication). How the elevated kinase is related to the postreceptor defect remains to be elucidated.

Muscle Soleus from fatty rats demonstrates about a 50% reduction in insulin binding [in cardiac muscle this reduction was ascribed to changes in the low-affinity binding site (23, 27)]. In vitro, the dose-response curve for insulin-stimulated glucose transport is shifted to the right in 10-week-old fatty rats but not in 6-week-old rats. In addition to the receptor deficiency, there is also a reduction in the maximum level of insulin-stimulated glucose utiliza-

tion, which suggests a defect distal to the binding of insulin (23). Similar defects are found in cardiac myocytes (27). With the in vivo euglycemic clamp technique, in 4- and 12-week-old fatty rats the curve of glucose utilization by muscle in response to insulin is shifted to the right; the maximal effect of insulin is unaltered in the 4-week-old but reduced in the 12-week-old rats (65). Insulin-induced tyrosine kinase is unaffected in 4-week-old fatty rats (24).

Adipose At 4 weeks of age the rats demonstrate elevated insulin-sensitive glucose transport associated with an increased number of glucose transporters as well as elevated tyrosine kinase activity (24, 43). At 6–10 weeks, rats have three times more insulin receptors than normal, with an associated elevated 2-deoxyglucose uptake (22, 23). At 12 weeks, others have shown reduced insulin sensitivity (65). By 20 weeks of age, the insulin receptor number is normal and insulin-stimulated glucose metabolism is markedly reduced (22). These results probably reflect changes associated with aging.

Brain In one study the concentration of insulin receptors and the tyrosine kinase activity were similar in fatty and lean rats (J. Shemer, A. Ota, D. LeRoith, submitted for publication). Figlewicz et al (30), however, found reduced insulin receptor number in olfactory bulbs from homozygous fatty and heterozygous lean animals, with no change in receptors from the cerebral cortex or hypothalamus.

MANIPULATIONS OF ZUCKER FATTY RATS In 5-month and 12-month fatty rats, dietary restrictions lead to normalization of body weight and amelioration of the hyperinsulinemia and insulin receptor deficiency (18).

In 12–15-week-old fatty rats that have normal liver insulin binding (12), starvation for 24 to 72 hours fails to increase insulin receptors in liver despite reduced plasma insulin levels. In contrast, when 16-week-old fatty rats with reduced liver insulin binding are fasted for 72 hours, insulin receptors increase (J. Shemer, A. Ota, D. LeRoith, submitted for publication). In addition, insulin receptors from heterozygous lean animals also increase following the acute fast. In the younger fatty animals with normal numbers of liver insulin receptors, repeated injections of streptozotocin are associated with reduced plasma insulin levels and elevated numbers of liver insulin receptors (19).

SUMMARY In contrast to the ob/ob mouse, the Zucker fatty rat only develops "down-regulation" of liver insulin receptors at approximately three to five months, despite hyperinsulinemia at an earlier age. In addition, postreceptor abnormalities exist at five months, though the relationship to alterations in

receptor tyrosine kinase are as yet undefined. Muscle demonstrates both down-regulation of insulin receptors and postreceptor defects. In contrast, in young fatty rats, adipocyte insulin receptor number and insulin action are enhanced. Thus age and tissue differences are important factors in studying the Zucker fatty rat as a model of obesity.

Wistar Kyoto Rat

Figlewicz et al (31) studied insulin receptors of liver and brain in the Wistar Kyoto rat that had the fatty gene from Zucker rats bred into it. They found results similar to their findings with Zucker fatty rats, namely insulin receptors of the liver, cerebral cortex, olfactory bulb, and lateral (but not medial) hypothalamus from both fatty and heterozygous lean rats are reduced in number relative to those in homozygous lean animals.

LA/N Corpulent Rat

LA/N corpulent rats inherit obesity as an autosomal recessive trait and appear to be a model for obesity and hyperlipidemia (8). They are hyperinsulinemic (6–7-fold higher insulin levels than in thin littermates) but normoglycemic, and they exhibit an approximate fivefold reduction in liver insulin receptor number compared to thin littermates whether fed sucrose or starch (8).

SHR/N Corpulent Rat

SHR/N corpulent rats inherit obesity as an autosomal recessive trait and have plasma insulin levels approximately 5–10-fold higher than thin littermates (57), but unlike their thin littermates, they do not become spontaneously hypertensive. In this obese strain, which may be a model of obesity associated with type II diabetes, there is a significantly reduced number of liver insulin receptors, an effect that is not modulated by dietary sucrose (6a; M. Adamo, J. Shemer, and D. LeRoith, unpublished observations).

AGED OBESE RATS

Insulin receptors and insulin action have been studied as rats age, when they become relatively obese and hyperinsulinemic. While the concentrations of insulin receptors in the aged rats are a subject of disagreement (15, 21, 59, 60, 62, 78), various investigators have consistently found impairment of insulin action. Thus, maximally insulin-stimulated glucose metabolism is lower in adipocytes from the older obese rats than in the younger, thin animals. This effect may be due to impaired intracellular glucose metabolism, as well as a receptor defect (59); a marked reduction in insulin-stimulated glucose transport attributable to a reduction in the number of intracellular glucose transporters has also been reported (21).

OBESITY IN HUMANS

Obesity in humans is defined by the increase over ideal body weight for a given age and sex. While most type II (NIDDM) diabetics are obese, most obese people are not diabetic. Simple obesity in the absence of diabetes is associated with hyperinsulinemia and reduced numbers of insulin receptors on most tissues studied (4, 5, 25, 64, 75). Furthermore, insulin dose-response curves on fat cells from these obese subjects reflect the receptor defect as well as postreceptor defects (64). Receptor tyrosine kinase activity is unchanged in liver and fat cells, while in muscle it is reduced (13, 14, 33). Short-term fasting (48–72 hr) is associated with improvement in receptor affinity (5), while long-term fasting (3–14 days) restores insulin receptor numbers (5, 25, 64). These changes are all associated with a reduction in hyperinsulinemia. Interestingly, despite the improvement in insulin receptor binding with fasting of obese subjects, the patients are still unresponsive to insulin (25, 64), although the biochemical basis for it may shift.

When obesity is associated with type II diabetes, there appears to be a further reduction in the number of insulin receptors in adipocytes (33), though, muscle and liver (cell surface) insulin receptor numbers were similar in obese controls and obese type II diabetics (13, 14). Insulin receptor tyrosine kinase activity is less in liver and adipocytes, but not muscle of obese type II diabetics versus obese nondiabetics (13, 14, 33).

DIETARY ALTERATIONS

Altered Dietary Carbohydrate

High-carbohydrate diets decrease insulin receptor numbers in human and most mammalian species studied (38, 52, 63, 69, 77), probably as a result of the hyperinsulinemia and resultant receptor down-regulation accompanying the high-carbohydrate diet. Interestingly, despite the reduced receptor number the high-carbohydrate diet is associated with markedly elevated insulin-stimulated glucose uptake and metabolism in rat adipocytes as well as increased insulin sensitivity in vivo (52, 63). This enhancement in insulin action after high-carbohydrate feeding despite reduced receptor number may be a consequence of elevated insulin receptor tyrosine kinase activity as observed in the liver of rats fed high-carbohydrate diets (34).

Altered Dietary Lipid

Feeding high-fat diets results in a reduction in the number of insulin receptors as well as in receptor affinity (7, 38, 46, 63, 77). Dietary cholesterol increases receptor number and reverses the effect of a high-fat diet (7). In contrast to high-carbohydrate feeding, high-fat diets result in a loss of sensitivity and

responsiveness to insulin stimulation of glucose uptake and metabolism, which reflects the receptor as well as postreceptor defects (46, 63).

Altered Dietary Protein

In goats a high-protein diet reduces the number of hepatic insulin receptors (38), while nitrogen restriction increases skeletal muscle insulin receptors without altering hepatic receptors (40, 41). In contrast, high-protein diets do not alter liver or muscle insulin receptors in rainbow trout (1).

FASTING AND REFEEDING OF NORMALS

In liver and adipocytes, 48–72 hr of fasting increases affinity and/or number of insulin receptors (3, 15, 29, 50, 61, 71, 81). Insulin action is nevertheless reduced, possibly because of decreases in receptor tyrosine kinase activity and/or insulin mediator generation (34, 71, 81).

In rat muscle insulin binding and action are affected in the same direction by fasting. Thus, Brady et al (10) found that a 48-hour fast increases both the number and affinity of soleus muscle insulin receptors and also enhances insulin-stimulated glucose metabolism through both receptor and postreceptor effects.

Refeeding of previously starved rats and chickens results in a reduction of insulin receptor number back toward control levels (29, 50, 71). Concomitantly, glucokinase, an insulin-sensitive enzyme, increases in rat liver (29), while in rat adipocytes, insulin-stimulated glucose metabolism is increased back toward that seen in ad libitum fed controls (50). Likewise, in chickens, refeeding after a 48-hr fast tends to restore receptor activity back toward that seen in ad libitum fed controls (71). In contrast, chicken muscle insulin receptor binding and tyrosine kinase activity were unaltered by fasting and refeeding (2a).

CONCLUSIONS

It is clear from the foregoing review that a wide variety of altered nutritional states affect both insulin receptors and cellular metabolic responses to insulin. Whether one considers the obesity and hyperphagia associated with genetic and acquired rodent models, the spontaneous obesity of rodents and humans, or experimentally induced alterations in the nutrient or caloric diet of normal animals and humans, it appears overall that the number of insulin receptors measured in in vitro binding assays is inversely related to the plasma insulin level resulting from the altered nutritional state. Thus, in conditions that might be simply described as "overnutrition," i.e. obesity, or high-carbo-

hydrate, -fat, or -protein feeding, concomitant or resultant hyperinsulinemia produces a down-regulation of cellular insulin receptors. Conversely, in conditions of "undernutrition," i.e. fasting or caloric restriction, the resulting reduction in plasma insulin levels increases the number of cellular insulin receptors. These simple relationships have a few exceptions, e.g. young obese Zucker rats.

It is important to recognize that the relationship of changes in insulin receptor number to insulin action is not straightforward. Thus, in obese models and some cases of overnutrition (e.g. high-fat feeding) the reduced insulin receptors play some, but not the entire, role in the resistance to insulin action. This is illustrated in those studies in which caloric restriction of various degrees ameliorates the hyperinsulinemia and increases insulin receptor numbers, but fails to improve insulin sensitivity or response, except in muscle. In other instances (e.g. fasting and high-carbohydrate feeding) the changes in insulin receptor number and insulin action actually occur in opposite directions, which suggests that nutrient availability or unavailability may supersede the insulin receptor status in determining the ultimate metabolic response to insulin presence. Superimposed on this is the apparent tissue variability in response, in which muscle, perhaps the preeminent target for insulin action, exhibits the closest correlation between insulin receptor number and action in states of altered nutrition. It is evident that future research will focus on the mechanism(s) of postbinding alterations in insulin action in altered nutritional states and, as suggested in this review, may reveal a role for the insulin receptor tyrosine kinase.

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