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COMMENTARY

GLUCAGON AND ADIPOSE TISSUE

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Although glucagon was discovered more than 50 years ago by Murlin and his coworkers, its importance in physiology and pathology has only recently been recognized. Its chemical structure is well established and consists of a single-chain polypeptide comprising 29 amino-acid residues with a total molecular weight of 3485. The amino-acid sequence is identical in many species thus far studied, including man, pig, rat, cow and camel, while the primary structure of glucagon in some avian species such as the duck and turkey presents only minor changes. The α_2 cells of the islets of Langerhans in the pancreas are the main source of glucagon. However, most recent studies have demonstrated the existence, maybe limited to some animal species like the dog or the pig, of a "glucagon" originating from the digestive tract (mainly the antrum of the stomach) and which behaves immunologically and physiologically exactly like pancreatic glucagon [1]. The origin of this material seems to be in specific cells of the digestive tract which are ultrastructurally indistinguishable from pancreatic α_2 cells. This "glucagon" originating from the digestive tract should not be confused with the so-called "gut glucagon-like immunoreactive material" or "gut GLI" which also originates from the gut, but whose molecular weight and immunoreactivity distinguish it from crystallized pancreatic glucagon.* For the time being, our analysis will be limited to pancreatic glucagon. The development in 1959 by Unger and his group of a radioimmunoassay method for glucagon [3] stimulated numerous studies of the physiological role of this hormone. These studies, as recently reviewed [4], provided conclusive evidence that glucagon plays a major role in the moment-to-moment regulation of blood glucose homeostasis. In fact, it has been undoubtedly demonstrated that a decrease in blood glucose markedly stimulates glucagon secretion and, conversely, that a rise in blood glucose is a potent inhibitor of glucagon secretion. In turn, glucagon stimulates glucose output from the liver by complex mechanisms involving inhibition of glycogen synthesis, stimulation of glycogenolysis and stimulation of gluconeogenesis (see review in Park and Exton [5]). It has only been shown recently that, in contributing to the hyperglycemia of diabetes, disorders of glucagon secretion also play a role in pathological processes. Somatostatin, a hypothalamic tetradecapeptide [6] of ovine origin, which can also be synthetized, inhibits growth hormone secretion [7, 8]; it is also a potent inhibitor of insulin [9] and glucagon [10] secretion. When infused in insulin-dependent (and therefore endogenous insulin-lacking) diabetic subjects, somatostatin induced a clearcut decrease in circulating glucagon and a parallel decrease in blood glucose [11]. The fact that a similar response occurred in a hypophysectomized diabetic patient demonstrates that these effects of somatostatin are independent of suppression of growth hormone secretion [11]. Next to the hepatocyte, it has been demonstrated that the adipocyte is also extremely sensitive to glucagon, which induces increased lipolysis. We shall consider here (1) the main characteristics of glucagon-induced lipolysis, (2) some of the factors which modify glucagon-increased lipolysis and (3) the physiologic importance of these observations.

GLUCAGON-INDUCED LIPOLYSIS

In several species, glucagon enhances the release *in vitro* of glycerol and free fatty acids (FFA) from pieces of adipose tissue or from isolated adipose cells but not always to the same degree (review in [12]). Rat, mouse and rabbit adipose tissues respond well to glucagon, avian adipose tissue is extremely sensitive to glucagon, while dog or human adipose tissue responds only under certain experimental conditions.

A number of structural modifications of the glucagon molecule can suppress or diminish its lipolytic activity. Assan and Slusher [13] have demonstrated that the 19–22 amino-acid sequence of the glucagon molecule is essential for its lipolytic activity and Rodbell and his group [14] have shown that de-histidine glucagon (2–29 amino-acid sequence of the polypeptide) is devoid of lipolytic activity even though it still binds effectively with the glucagon receptor of the plasma membrane.

The minimal effective concentration of glucagon needed to induce glycerol release *in vitro* is 0·002 μg/ml using pieces of rat adipose tissue, 0·001 μg/ml with rat adipose cells, 0·001 μg/ml with chicken adipose tissue pieces and 0·0001 μg/ml with chicken adipose tissue cells. These concentrations should be compared with the average plasma glucagon concentrations, which reach 300–500 pg/ml after moderate stimulation and up to 1500 pg/ml under maximal stimulation (or certain pathologic conditions).

* The nomenclature of this field is particularly confusing. Unger and his coworkers have recently proposed to call "*gut glucagon*" the material which could not be differentiated from pancreatic glucagon by biologic, physiochemical and immunometric techniques. They have proposed the term "enteroglucagonoid" [2] (former "gut glucagon-like immunoreactive material") to designate the other substances present in the gut and which interfere, in an immunoassay, with the reaction between the labeled glucagon tracer and glucagon antibodies.

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Simultaneous measurements of net changes in glycerol and FFA in the adipose tissue itself and in the incubation medium, or the use of radioactive tracer procedures, have demonstrated that when lipolysis is stimulated by glucagon the calculated rate of esterification (or re-esterification) of FFA also increases [12]. As demonstrated below, the rate of (re)esterification can be altered by various modifications of the experimental conditions, thus modifying greatly the amount of FFA which are effectively mobilised by glucagon-stimulated lipolysis. The stimulation of lipolysis by glucagon is accompanied by a marked increase of glucose uptake and oxidation. Using C_1- and C_6-labeled glucose, it has been shown that the increase in oxidation of C_6 is proportionally greater than that of C_1 oxidation. This indicates that the glycolytic pathway is stimulated to a greater degree by glucagon than is the shunt pathway in the adipose cell. Part of the glucose that is utilized by the adipose cell exposed to glucagon provides the α-glycerophosphate necessary for the (re)-esterification process. This is essential since it is generally accepted that white adipose cells are devoid of any significant amount of glycerokinase and are therefore unable to phosphorylate glycerol directly to produce the α-glycerophosphate which is a prerequisite for fatty acid (re)-esterification. Experiments based on the use of various inhibitors of lipolysis, have, however, clearly shown that glucagon can also stimulate the entry and overall oxidation of glucose by a mechanism which is distinct from its lipolysis-stimulating mechanism [15].

The mechanism by which glucagon is thought to stimulate lipolysis has been extensively studied by Rodbell and his coworkers [14]. As a first step, the hormone binds to a specific protein receptor situated on the outer surface of the adipose cell plasma membrane. As a consequence of the glucagon-receptor interaction, a cascade of subsequent events will ultimately result in the stimulation of lipolysis (Fig. 1). These events involve successively adenylyl-cyclase, protein-kinase, cyclic AMP, hormone-sensitive triglyceride lipase and finally triglyceride breakdown. The intimate mechanisms linking those events are only partially known and are out of the scope of the present review.

SOME FACTORS WHICH MODIFY GLUCAGON-INDUCED LIPOLYSIS

Large rat adipocytes exhibit a marked resistance to the lipolytic action of glucagon when compared with small cells [16]. Since, in these experiments, small fat cells were prepared from young animals and large cells from adult animals, it is difficult to decide whether the resistance to glucagon is linked to the *size* of the cell or to the *age* of the animal. The underlying mechanism for the relative insensitivity to glucagon remains unclear. Studies of Livingston *et al.* [17] indicated clearly that large cells bind less ^{125}I-labeled glucagon than small cells. The diminished binding is not a consequence of increased glucagon degradation since similar amounts of the tracer were degraded by both cell types. Surprisingly, the decrease in ^{125}I-labeled glucagon binding did not parallel the decrease in the lipolytic response to glucagon. Therefore, diminished binding explains only in part the marked resistance to glucagon of large rat fat cells. A possible explanation may be found in the fact that phosphodiesterase activity, which degrades cyclic AMP, is greater in large cells than in small cells [18]. The lipolytic action of glucagon is inhibited by insulin in most animal species but not in birds. Using rat adipose tissue pieces incubated in a medium containing 100 mg% of glucose, the lipolytic action of glucagon prevails over the antilipolytic effect of insulin only if the insulin to glucagon molar ratio is equal to or below 0·2 [19]. This suggests that, on a molar basis and in this particular species, the antilipolytic action of insulin is about five times more potent than the lipolytic effect of glucagon. In birds, there is general agreement that insulin does not inhibit the lipolytic effect of glucagon which is particularly potent in avian species. In birds, as well as in other species, the lipolytic action of glucagon is markedly reduced, and at high concentrations completely abolished, by

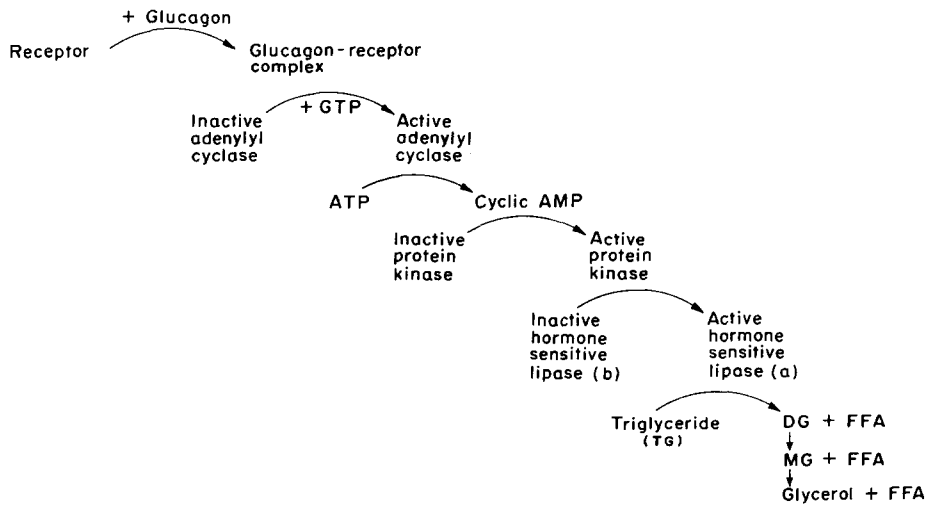
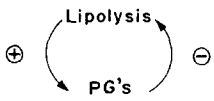


Fig. 1. The glucagon-induced lipolytic cascade (adapted from Steinberg and Huttunen [62]). GTP = guanosine tri-phosphate; ATP = adenosine tri-phosphate; DG = diglyceride; MG = monoglyceride; FFA = free fatty acid.

the prostaglandin PGE_1 (review in [20]). This action has been attributed to an *inhibition* of cyclic AMP formation by PGE_1, an effect which has been demonstrated in isolated fat *cells* (in contrast to the unexpected *stimulation* of adenylylcyclase activity of *intact fat* pads exposed to PGE_1). Since it has been demonstrated that hormone- or nerve stimulation-induced lipolysis is accompanied by the release of prostaglandins in the incubation medium it has been suggested that the local release of prostaglandins during lipolysis may modulate the action of the original stimulus by a negative feed-back mechanism [21, 22].

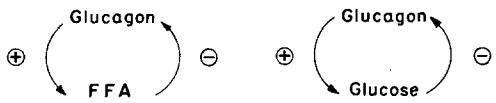


Such a concept was supported by the findings of Illiano and Cuatrecasas [23], who demonstrated that blocking endogenous prostaglandin biosynthesis with indomethacin or competitively antagonizing the action of prostaglandins by specific inhibitors, such as 7-oxa-13 prostynoic acid or SC-19220, a dibenzoazepine hydrazide, resulted in enhanced adrenaline- or ACTH-induced lipolysis. However, various investigators [24–26], using similar *in vitro* protocols, were unable to confirm these results. In a recent study [20], we investigated the effect of L8027, a pyridylindolyl ketone which is a potent inhibitor of prostaglandin biosynthesis, on basal and glucagon-stimulated adipose tissue lipolysis in rats. Chronic administration of this compound to rats (50 mg/kg per day for 15 days) resulted in a significant increase in lipolysis when adipose tissue of treated rats was subsequently incubated in the presence of glucagon. This finding supports the concept that endogenous prostaglandins may be concerned with the regulation of glucagon-induced lipolysis. This idea is reinforced by the observation that chronic treatment with L8027 enhanced exercise-induced lipolysis, a situation in which glucagon may be involved (see below). Confirmation of the theory that endogenous prostaglandins modulate glucagon-induced lipolysis requires demonstration that L8027 inhibits prostaglandin synthesis in adipose tissue itself; up till now, these effects of L8027 have been observed only in homogenates of sheep seminal vesicles, where prostaglandin biosynthesis is particularly active. This obligatory control experiment is now the aim of future research in our Laboratory. Other pharmacological factors affecting glucagon-induced lipolysis include theophylline, a classical phosphodiesterase inhibitor, which enhances the action of glucagon [27] and imidazole, a phosphodiesterase activator, which reduces the effect of glucagon [28]. Denervated white rat adipose tissue incubated *in vitro* responds to glucagon by a stimulation of lipolysis which is quantitatively similar to the one observed in normally innervated adipose tissue [29]. In contrast, glucagon-induced FFA release from denervated adipose tissue is significantly reduced. Since denervation is associated with a significant increase in glucose uptake, the decrease in FFA release has been attributed to a relative increase in FFA (re)esterification in denervated adipose tissue [29]. We have suggested that this mechanism may play a role in explaining the relative weight increase of denervated adipose tissue [29], a well known phenomenon. The lipolytic response to glucagon is reduced, or even completely abolished, by previous hypophysectomy or adrenalectomy and in hypothyroid animals. The mechanism by which hypophysectomy produces this effect is not completely understood; recent data of Gorin and Goodman [30] suggest that decreased protein-kinase activity may be, at least partly, responsible for the decreased cyclic AMP-induced activation of lipase found in adipose tissue of hypophysectomized rats. Regarding the adrenals, it is generally accepted that the "permissive effect" of glucocorticoid is required for a normal response to lipolytic hormones. The biochemical lesion or lesions induced by adrenalectomy in adipose tissue are still poorly understood (see discussion in Werner and Löw [31]). The disability of hypothyroid animals with regard to glucagon-induced fat mobilization from adipose tissue is apparently not linked to a defect in the adenylyl cyclase system; it has been recently attributed to the particularly high activity in hypothyroid animals of a membrane associated, high affinity, cyclic AMP phosphodiesterase [32].

PHYSIOLOGIC IMPORTANCE OF THE GLUCAGON-INDUCED LIPOLYSIS

As emphasized above, glucagon has been demonstrated to be one of the most potent lipolytic hormones when studied *in vitro* using adipose tissue or cells from several animal species. When infused or injected *in vivo*, glucagon increases peripheral plasma glycerol or free fatty acid (FFA) levels under various experimental or clinical conditions (review in [12]). This situation is encountered (1) when glucagon is injected in birds [33] (a species in which the extreme glucagon-induced mobilization of FFA from the adipose tissue stores leads to an increased liver uptake of FFA and a secondary marked accumulation of liver triglycerides), (2) when glucagon is infused at low concentrations into the portal vein of anesthetized dogs who were fasted for 18 hr [34] and (3) in man, when minimal amounts of glucagon are infused in low doses in normal subjects after 3 days of total starvation [35] or in insulin-dependent diabetics [36]. On the contrary under other experimental conditions, glucagon has been reported to induce a decrease in plasma FFA. This paradoxical situation can be explained by numerous factors [12] which include (1) the possible contamination of the glucagon preparation by insulin, (2) the hyperglycemic response to glucagon, and (3) the insulinogenic effect of glucagon. Strong arguments in favor of the physiologic relevance of glucagon-induced lipolysis have been brought by several experiments *in vivo* and *in vitro* demonstrating that FFA, the final product of lipolysis, were indeed able to modulate glucagon secretion. In the dog, an increase in plasma FFA obtained by the simultaneous infusion of triglycerides and heparin, depresses peripheral plasma glucagon levels [37] and, conversely, a decrease in plasma FFA, induced by the administration of various antilipolytic agents, is followed by a clearcut increase in pancreatic glucagon production [38, 39]. Similar results have been obtained in the duck [40] and, under certain conditions, in man. It should, however, be mentioned that investigations in man gave conflicting results: while Hicks *et al.* [41] did not find that glucagon

variations were induced by changes in plasma FFA, Gerich *et al.* [42] as well as Andrews and Blackard [43] showed the opposite. In recent investigations, Quabbe and Luyckx [44] have clearly demonstrated that the rise in peripheral plasma glucagon observed during a nicotinic acid-induced fall in plasma FFA was inhibited by graded infusions of glucose, an effect which was related precisely to the amount of glucose infused. All these observations suggest that circulating FFA levels are controlled by a negative feed-back mechanism in which FFA regulate the release of the lipolytic agent, i.e. glucagon, in the same manner in which glucose levels affect glucagon secretion [39].



This concept is also supported by the data of experiments *in vitro*. Glucagon release is markedly inhibited when isolated guinea-pig islets of Langerhans are incubated in the presence of octanoate and palmitate [45]. In this system, relatively low concentrations of free fatty acids markedly inhibit glucagon release, in contrast to the slight inhibition induced by high concentrations of glucose under these experimental conditions. In addition, the inhibition of glucagon release by octanoate was not affected by varying the concentration of glucose in the incubation medium, suggesting that fatty acid levels may be more important than glucose concentrations in the regulation of glucagon release from the guinea-pig pancreas *in vitro*. Extensive studies of Luyckx [38, 46], using the isolated perfused rat pancreas, have confirmed that there is a marked inhibition of glucagon release when the perfusion medium contains high levels of FFA. Similarly, the unequivocal rise in glucagon release observed in this system when the concentration of glucose in the perfusion medium falls from 80 to 25 mg%, is significantly reduced in the presence of high concentrations of FFA. Thus, the concept of glucagon as a "hormone of glucose need" as proposed by Unger [47] can be extended to that of glucagon as a "hormone of fuel need" [48] or of "nutrient need", based on the above-mentioned observations which show that glucagon is able to promote not only the mobilization of glucose from liver stores but also that of FFA from adipose tissue stores, and that both glucose and FFA have been demonstrated to modulate glucagon secretion via a negative feed-back system.

We would like to end this commentary by considering the following question: Are there physiological or pathophysiological conditions under which glucagon acts as an important lipid mobilizing hormone? We shall restrict our answer to the analysis of a few conditions in which there undoubtedly exists a "fuel need" and in which glucagon may reasonably be involved. These include fasting, muscular exercise, adaptation of mammals to the extra-uterine life and diabetic keto-acidosis.

(1) Fasting is *par excellence* a condition of fuel need. It is associated with an unequivocal rise in both plasma FFA and plasma glucagon (review in [4]). Recent investigations of Koerker *et al.* [49] in fasting baboons have indicated that FFA and glycerol levels cycle in phase with glucagon levels with a significant correlation, thus suggesting that the two events, glucagon secretion and lipolysis, are either causally related, or else share a common (neural ?) regulatory mechanism. Since fasting is accompanied by an inhibition of insulin secretion, the insulin/glucagon molar ratio (I/G) in peripheral blood is substantially decreased. In the experiments of Luyckx in rats, it falls from 6 to 8 in the postabsorptive state to values ranging from 1·5 to 2 after 2 or 3 days of starvation [39]. This ratio would seem to favor adipose tissue lipolysis but we have to recall here that *in vitro*, when rat adipose tissue is incubated with various concentrations of glucagon and insulin, lipolysis increased above basal values, only when the I/G molar ratio was below 0·2 [19]. A value as low as this is rarely observed under physiological conditions. Nevertheless, if one recalls that insulin is the major, if not the only* circulating antilipolytic hormone, glucagon is far from being the only circulating lipolytic hormone. Therefore we suggest to consider the following ratio as the one physiologically important as far as adipose tissue lipolysis is concerned:

$$\frac{\text{antilipolytic hormones}}{\text{lipolytic hormones}} = \frac{\text{insulin (+ other antilipolytic hormones ?)}}{\text{glucagon + other lipolytic hormones}}$$

On this basis, we consider that glucagon can reasonably be assumed to be a contributory factor to lipolysis under fasting conditions in a given species when: (1) adipose cells of the species considered are sensitive to minimal levels of glucagon; and (2) fasting induces an increase in peripheral plasma glucagon levels in that species. While these phenomena occur in rats and in birds, there is no definite proof that they occur in other species, including man.

(2) *Muscular exercise* is accompanied by a striking rise in plasma glucagon [50–54]. There is evidence that the excercise-induced rise in plasma glucagon may be due to at least two basic mechanisms [39]: (1) a stimulation of the adrenergic system which in turn stimulates the β adrenergic receptors of the α_2 cell resulting in glucagon release ("neural stimulation") and (2) a decrease in circulating glucose ("metabolic stimulation"). Extensive studies from our Laboratory [51–54], using rats forced to swim as a model of physical exercise, have demonstrated that, under this particular condition and in that species, "neural stimulation" is the predominant factor [53, 54]. With more strenuous exercise [39, 55] "metabolic stimulation" may also become important. In a series of studies, done in collaboration with Luyckx, we investigated the role of glucagon in exercise studying various factors which control glucagon release, adipose tissue FFA mobilization or both [53, 54]. Some

* Other possible substances, though the status of hormone in the traditional sense has not yet been accorded them, are the prostaglandins which are among the most active antilipolytic biological substances.

Table 1. Comparison of the changes in plasma glucagon and plasma FFA as observed after muscular exercise (forced swim) in rats

(I) Simultaneous rise in plasma glucagon and plasma FFA
Normal, untrained rats [51–54]
Normal, trained rats [54]
Medullo-adrenalectomy [54]
d-Propranolol (5 mg/kg) [54]
(II) Normal rise in plasma glucagon but no rise in plasma FFA
Adrenalectomy [53]
Hypophysectomy [53]
Phentolamine (5 mg/kg) [53]
(III) No rise in plasma glucagon but normal rise in plasma FFA
Immunosympathectomy [54]
d,l-Propranolol (5 mg/kg) [53]
Pindolol (0·1 mg/kg) [54]

of our findings are summarized in Table 1 which clearly demonstrates that the exercise-induced glucagon rise may be dissociated from the exercise-induced FFA rise. The fact that FFA levels may be increased with exercise while glucagon levels remain at baseline values suggest that glucagon is not essential for adipose tissue FFA release during exercise but does not exclude the possibility that glucagon, during the period when its levels are raised, could contribute along with other factors to the rise in FFA observed as a consequence of exercise. Exercise-induced FFA rise is a complex phenomenon which is the resultant of various changes induced by exercise [51]: (1) a stimulation of lipolysis induced by circulating lipolytic hormones whose secretion is stimulated (besides glucagon, circulating catecholamines, growth hormone and others) or by the local release of noradrenaline from nerve endings in adipose tissue, (2) a decrease in the "antilipolytic tone" essentially due to the exercise-induced decrease in circulating insulin and (3) a decrease in esterification or reesterification of FFA within adipose tissue [56], a factor to which contribute the decreased circulating levels of insulin, the lesser availability of glucose for adipose tissue, the increased circulating levels of corticosterone and, perhaps, the release of catecholamines from nerve endings within adipose tissue. The multiplicity of factors involved in metabolic regulation during exercise and the interplay between them satisfactorily explains the observation that when only one of these factors has been neutralized, the final result (i.e. FFA mobilization) may be unaffected. On the contrary, when the FFA mobilization itself is experimentally blocked (unresponsiveness of adipose tissue in adrenalectomized or hypophysectomized animals for instance), the circulating hormones (glucagon for instance) may be increased without showing their effects.

(3) The *adaptation of newborn mammals to extra-uterine life* is another situation in which an acute fuel need is present. Evidence is now available showing that glucagon is already secreted at birth and that its level increases during the first hours of the extra-uterine life. Thus far, this has been demonstrated in the rat [57] and in the human newborn [58]. Several lines of evidence indicate that glucagon is involved in the release of glucose from the liver at this crucial period of life. In order to prove that fatty acid mobilization from adipose tissue, a process which is extremely active in the newborn (refs. [59]), results at least partially from increased glucagon, it must be demonstrated that adipocytes* in the neonate are sensitive to minimal concentrations of glucagon.

(4) *Diabetic ketoacidosis* is a situation which is associated with high circulating levels of FFA and extremely high levels of plasma glucagon [60, 61]. Several lines of evidence indicate that the total lack of insulin plays an important role in the hyperglucagonemia (it has been suggested that insulin may be an important factor in permitting glucose entry into the α_2 cell and therefore allowing glucagon suppression). The concept that glucagon may play a role in the FFA mobilization of diabetic ketoacidosis is supported by the findings of Liljenqvist *et al.* [36] who demonstrated that glucagon, at relatively high concentrations ($1{\cdot}3 \times 10^{-9}$ M), is capable of stimulating lipolysis and ketogenesis in insulin-dependant diabetic man.

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* The simple *presence* of white adipose tissue must also be considered; the newborn rat for instance is known to be very poor in white adipose cells.

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