ENDOCRINOLOGY OF ADIPOSE TISSUE AND FAT CELL METABOLISM

by

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In the United States, using current husbandry practices, excess adipose tissue is accumulated in the three major meat-producing mammalian species—cattle, sheep, and swine. Much of this adipose tissue is essentially waste for the meat industry and of little economic value [2]. Although adipose tissue has many metabolic sequences in common with other tissues, e.g., carbohydrate and protein metabolism, its main function is to synthesize and store fat during periods of caloric excess, to provide energy through mobilization of fatty acids during periods of caloric deprivation, and to insulate the vital core of the animal from the environment. It should be emphasized at the outset that the current concept of adipose tissue is of a highly active tissue in a state of continued metabolic flux in contrast to the older view of a static tissue acting solely as an energy sink.

This review will be restricted to a discussion of lipid metabolism by white adipose tissue, the depot fat. It will not attempt to be exhaustive and will emphasize the metabolism of the major mammalian species raised for meat production, i.e., swine, cattle and sheep. Adipose tissue metabolism in these species has recently been reviewed [2] and particularly with emphasis on the ruminant [6, 8].

Since the majority of research has utilized an *in vitro* approach, one should be cognizant of the difficulty in translating *in vitro* experimental results to *in vivo* function. *In vitro* experiments generally indicate the capacity of a tissue to function but usually bypass the intricate and interconnected control systems that function *in vivo*.

Lipid Deposition. Long-chain fatty acids, the major energy containing constituents of triglyceride, the depot storage material, are either provided by the diet or synthesized by the animal. In post-weaning porcine, bovine, and ovine animals, raised under common husbandry conditions, have little fat in the diet and, consequently, *de novo* synthesis is the major source of long-chain fatty acids. In suckling animals the fat content of milk is relatively high and, consequently, dietary supply is relevant. In swine [54] and both of the ruminant species [7], the adipose tissue is the primary site for synthesis of fatty acids.

The major endocrine influence on fatty acid synthesis in adipose tissue is insulin. Among several observed insulin effects are an enhancement of glucose transport into the cell coupled with increased utilization of glucose for oxidation, fatty acid, and glyceride-glycerol synthesis and glycogen synthesis [25]. The insulin effects seem to result from the interaction of the hormone with a specific cell membrane receptor. The hormone-receptor complexation appears to be primary to the observed stimulation of sugar transport, although it is not clear how these processes are coupled. It is also not apparent whether other insulin stimulated functions are the direct result of insulin action or are secondary to increased intracellular glucose [25, 27].

Insulin effects on fatty acid synthesis are most dramatically observed in diabetic animals wherein carbon flux through the pathway as well as enzyme activities associated with carbon flux (citrate cleavage enzyme, acetyl-CoA carboxylase, fatty acid synthetase) and the generation of reducing equivalents (malate and hexose monophosphate shunt dehydrogenases) are all depressed with a return toward normal after injection of insulin [38, 39]. Insulin stimulates carbon flux to fatty acids and particularly the activity of two enzymes, pyruvate dehydrogenase and acetyl-CoA carboxylase, when incubated *in vitro* with rat adipose tissue [18, 39]. A dramatic *in vitro* effect of insulin may be observed in the differentiating adipocyte in culture (3T3-L1 cell line) wherein the cultured cells markedly increase substrate incorporation into lipids and accumulate lipid after confluence. In the absence of insulin in the culture medium, little differentiation is seen [26].

Recently, intracellular insulin receptors and the influx of intact insulin molecules into cells have been demonstrated [27]. Although the exact role of intracellular insulin remains to be elucidated, it may be important in some of the observed metabolic effects.

The stimulation of fatty acid synthesis upon incubation of adipose tissue with insulin is quite different among species [25]. The *in vitro* stimulation of fatty

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acid synthesis from glucose by bovine insulin using the same incubation medium was 290, 94, 61, and 67 percent for rat, guinea pig, rabbit, and hamster epididymal adipose tissue, respectively [19]; the most studied species, the rat, is the most responsive. These divergent responses to insulin cannot be attributed to rates of glucose metabolism since the rat and guinea pig have similar rates. The type of insulin used also is not the sole cause, since ovine and bovine insulin did not increase fatty acid synthesis in quinea pig or rabbit adipose tissue, nor, did rabbit insulin markedly stimulate rabbit tissue, although it was quite effective with rat adipose tissue.

Individual fat depots in the same animal may respond differently to *in vitro* insulin [25]. At least some of the differences probably result from distinct rates of growth or maturation of the various depots and, consequently, from cell size differences. Large rat adipocytes are generally more refractory to insulin stimulation than smaller adipocytes [20, 65].

Under usual husbandry conditions, swine are fed high carbohydrate diets, and glucose is probably the major precursor for the carbon atoms of fatty acids. Porcine insulin is generally added to *in vitro* incubations of swine adipose tissue when fatty acid synthesis is measured [1, 36, 41, 44]. However, in those studies where *in vitro* insulin effects have been measured via deletion and addition to the medium, only marginal stimulation of fatty acid synthesis from glucose has been observed [14, 53]. Glucose incorporation into lipids in backfat from pre- and post-weaned pigs incubated with 1 or 20 mM glucose was stimulated from 0 to 50 percent by porcine insulin additions between 0.1 and 100 mU/ml. These marginal insulin effects were observed using both tissue slices and isolated cells [44]. In one report of insulin stimulation of glucose incorporation into total lipids and fatty acids in swine [13] the effects were more obvious at lower glucose concentrations and were related to the insulin level used. The generally small *in vitro* insulin effects with swine adipose tissue ($< 50\%$) should be contrasted to the large insulin stimulation observed with rat adipose tissue ($> 200\%$).

The marginal *in vitro* effects of insulin on lipid synthesis in swine adipose tissue are unexpected in this species with extensive fat deposition. The tissue is definitely sensitive to insulin since alloxan diabetic pigs have very low rates of adipose tissue fatty acid synthesis [62]. Injection of insulin for several days restored the rates toward normal, whereas, upon withdrawal of the injected insulin, the rates declined to the low diabetic levels.

The role of insulin in lipid deposition in swine is far from clear because of the discrepancy between *in vitro* and *in vivo* effects. Swine respond to oral or intravenous glucose administration with an increase in plasma insulin and a decrease in plasma glucose [40, 76]. The basal plasma insulin is about 10 μU/ml and is increased to $\leqslant$ 100 μU/ml after glucose infusion. In contrast, the small *in vitro* effects are observed at 1 to 100 mU insulin/ml. The low *in vitro* response possibly results from tissue changes after removal from the animal, inactivation or absorption of insulin in the incubation flask, or may indicate a necessary role for insulin to maintain the capacity to synthesize fatty acid with a lesser role in the short-term regulation of fatty acid production.

In ruminant organisms, the major carbon precursor of long-chain fatty acid synthesized in adipose tissue is acetate produced by the rumen microflora [7]. Glucose is not readily incorporated into fatty acids by adipose tissue obtained from several different depots from either young or older ovine or bovine animals [30, 34]. Glucose is, however, a ready source of glyceride-glycerol and is consequently incorporated into total lipid [30, 79]. The incorporation of acetate into fatty acids is considerably enhanced by the addition of glucose plus insulin to the medium [28, 34, 79]. Consequently, insulin has routinely been added to *in vitro* ruminant adipose tissue incubation media [30, 56, 75].

The systematic assessment of the effect of insulin on the incorporation of acetate into fatty acids or of glucose into lipids has been studied by several groups [5, 42, 75]. Generally a modest increase ($< 100\%$) in incorporation of substrate into lipid was observed in the presence of insulin; however, one author [75] indicated no effect of exogenous insulin in ovine adipose tissue from animals at several ages. Two studies, one with bovine [79] and one with ovine [42] adipose tissue indicated an increase in glucose incorporation into glyceride-glycerol that was related to the insulin concentration. The bovine work also indicated stimulation of acetate incorporation into fatty acids by insulin, but only one insulin level was used.

The ruminant is responsive, *in vivo*, to stimuli that increase plasma insulin. In sheep, the plasma insulin was rapidly increased to > 100 μU/ml by intravenous propionate infusion and less rapidly to > 200 μU/ml by glucose infusion [10]. Intravenous insulin also increased the uptake of glucose by *in vivo* perfused adipose tissue of sheep [35].

As with swine, *in vitro* ruminant adipose tissue fatty acid synthesis was only moderately increased in the presence of insulin. Again, no gross defect seems to appear in the *in vivo* release of insulin to the plasma

upon appropriate stimulation. An explanatory hypothesis indicates that the low response of the herbivorous ruminant animal's adipose tissue to insulin may be an evolutionary adaptation to the essentially continuous eating patterns and nutrient supply from the rumen in these species [25]. In contrast, carnivores must respond to the intermittent food supply by a large increase in anabolic pathways and consequent energy storage at times of food supply. The omnivorous porcine animal also eats rather continuously and has a slow gut transit time so that the relative insulin insensitivity would be compatible with the hypothesis. It is not clear how the omnivorous and somewhat continuously eating rat fits with this hypothesis since its adipose tissue responses to insulin are so great.

Insulin may have a direct effect on the synthesis of triglyceride since palmitate incorporation into glyceride fatty acid is stimulated *in vitro* in rat epididymal adipose tissue by insulin. This stimulation is not as great as that observed for glucose incorporation into lipids but apparently is independent of insulin effects on glucose transport. Glycerophosphate acyltransferase activity (one of the enzymes in the pathway to triglyceride) is stimulated after *in vitro* incubation with insulin [64]. No hormonal effects on the synthesis of adipose tissue triglyceride in porcine, ovine, and bovine animals appear to be reported. Activities of several enzymes of the pathway for esterification of adipose tissue fatty acids are decreased during starvation in swine [71]. Although this result suggests regulation by insulin (decreased during starvation), it is only suggestive and could be secondary to effects on carbohydrate metabolism.

A major enzyme in the utilization of circulating lipid as a source of fatty acid for depot synthesis is lipoprotein lipase. The enzyme operates at the capillary wall to cleave the impermeable triglycerides to fatty acids that will cross the cell membrane to be incorporated into complex lipids [61]. In rat [9] and swine [70] adipose tissue, the lipoprotein lipase activity is decreased during starvation. The decrease in the rat enzyme activity was thwarted by intraperitoneal injection of insulin indicating a role of this hormone in enzyme maintenance. The bovine adipose tissue lipoprotein lipase activity was modestly increased after *in vivo* insulin injection and more definitively increased by *in vitro* incubation with the hormone [60].

Lipid Mobilization. Most stimulation of lipolytic activity is thought to occur via an interaction of an agonist (usually a hormone) with a specific adipose tissue membrane receptor resulting in the stimulation of adenyl cyclase and consequently an increase in cyclic AMP production. Activation of protein kinase by cyclic AMP leads to an activation of hormone-sensitive lipase by phosphorylation and subsequently to increased lipolysis with release of fatty acids and glycerol [43, 73]. The level of cyclic AMP, and consequently the lipolytic rate, may also be controlled by the activity of a phosphodiesterase enzyme that converts cyclic AMP to the inactive AMP.

Lipolysis Stimulated by a Number of Agents

Lipolysis is potentially stimulated by a number of agents [63]; among them are catecholamines, glucagon, pituitary trophic hormones (thyrotropin, adrenocorticotropin, somatotropin), and other pituitary factors (α and β melanocyte stimulating hormone, vasopressin). It may also be stimulated by methylxanthine type compounds, e.g., theophylline and caffeine, that inhibit phosphodiesterase. However, not all agents are effective in all species. For example, epinephrine stimulated lipolysis in adipose tissue from many species but not in rabbit nor guinea pig, whereas glucagon-stimulated in rabbit but not in dog. Each species apparently has its own spectrum of *in vitro* lipolytic agonists [52, 59, 63]. As with much biomedical research, the rat is the favorite model in lipolysis. Unfortunately, rat adipose tissue is receptive to a greater variety of lipolytic agonists than most other species.

Extensive reviews of substances that stimulate or inhibit lipolysis, including many pharmacological agents have appeared [23]. In particular, the catecholamine molecule has undergone detailed refinement to maximize the activity and the specificity for adipose tissue [24]. Historically, the use of a variety of agonists and antagonists (mostly synthetic) allowed the division of catecholamine effects into two receptor classes, α and β [reviewed in 23]. For example, contraction of the spleen is considered an α effect and heart contraction or tracheal relaxation a β effect. Subsequently, the β receptor has been divided into two subclasses, the β_1 receptor represented by heart contraction and the β_2 receptor represented by tracheal relaxation. Rat adipose tissue has been considered to be controlled by a β_1 receptor [23], although there is some question about this designation since several synthetic compounds considered to be β_2 specific agonists stimulate lipolysis in this species [24, unpublished observations].

The two major antilipolytic hormones are insulin and the prostaglandins (especially PGE). Insulin has both *in vitro* and *in vivo* antilipolytic activity at concentrations even lower than those needed to stimulate glucose transport [43, 73]. Although this hormone is

quite effective, the mechanism of action is not apparent. Evidence that insulin acts by control of cyclic AMP levels is inconclusive for both inhibition of synthesis and for stimulation of breakdown. The evidence for a physiological role of prostaglandins in the control of lipolysis is unclear since the circulating levels appear to be too low to be effective. These compounds may function as intracellular regulators.

Adipose tissue obtained from several different fat depots, both ovine and bovine ruminants, is stimulated to release fatty acids and/or glycerol when incubated *in vitro* with the catecholamines, epinephrine or norepinephrine [21, 22, 42, 51, 57, 80, 81]. The adrenergic response has not been characterized by use of various agonists and antagonists so that the receptor type cannot be classified. Epinephrine and norepinephrine produced the same lipolytic response in bovine adipocytes, but the level of epinephrine required was about an order of magnitude less than the level of norepinephrine [80]. This order of response would suggest a β_2 type of receptor in opposition to the rat where norepinephrine is more effective than epinephrine. The physiological effect of catecholamines regarding stimulation of ruminant lipolysis is unclear because the *in vivo* response of the ovine and bovine animal to intravenous norepinephrine or epinephrine was rather small [35, 67].

Lipolysis by ruminant adipose tissue (*in vitro*) is also stimulated by dibutyryl cyclic AMP, an analogue of cyclic AMP [21], by cyclic AMP [66] and by the phosphodiesterase inhibitor, theophylline [21, 22]. Theophylline, as expected, potentiated the response to epinephrine. Neither glucagon nor β-estradiol stimulated lipolysis [22]. The lack of agonistic activity by β-estradiol is surprising since greater lipolytic rates have been measured in late compared to early gestation [22, 51]. In late gestation, adipose tissue shifts to high rates of fatty acid generation with increased fatty acid release for transport to the mammary gland for milk-fat synthesis.

Antilipolytic effects of insulin were reported both *in vitro* and in adipose tissue isolated from insulin injected cows [80], although other workers did not observe antilipolytic activities of insulin [51]. Another potential antilipolytic compound, the prostaglandin PGE_2, does not inhibit *in vitro* bovine lipolysis [21].

Swine Adipose Tissue Lipolysis

Catecholamines stimulate lipolysis in swine adipose tissue during *in vitro* incubation [45, 49, 68, 77] and after *in vivo* infusion [4, 15, 29, 33, 55, 69]. Recently, the *in vivo* response to norepinephrine was used to demonstrate a greater lipolytic potential in lean Pietrain swine compared to more obese Large White swine [78].

The swine adipose tissue catecholamine receptor appears to be of the β-type as demonstrated *in vitro* with several agonists and antagonists [45]. This is confirmed *in vivo* since the norepinephrine stimulated fatty acid release is antagonized by the β-blocker, propranolol but not by the α-blocker, phentolamine [78]. *In vivo* release of fatty acids is also markedly stimulated by infusion of isoproterenol, a β agonist (unpublished observations). This receptor has not been further classified into a β_1 or β_2 type, although the order of effectiveness of the agonists, isoproterenol $>>$ norepinephrine $>$ epinephrine [45] would suggest a β_1 type receptor as would the more effective antagonistic effect of practolol (β_1) compared to butoxamine (β_2) [unpublished observations].

Swine adipose tissue lipolysis (*in vitro*) was very marginally stimulated by somatotropin and adrenocorticotropin but not by cyclic AMP or glucagon. The marginal stimulation by the two trophic hormones and the negative responses were similar at a number of ages between birth and day 80, postpartum, when tested over an extensive range of drug concentrations. The tissue was stimulated by dibutyryl cyclic AMP at all ages. Theophylline only slighty stimulated lipolysis at any age, but in the presence of a small amount of epinephrine it was maximally effective. Theophylline greatly enhanced the marginal effects of somatotropin and adrenocorticotropin as well as the effect of epinephrine but had no effect on the negative glucagon response [45, 47, 49, 50]. Stimulation of lipolysis by porcine somatotropin after long *in vitro* incubation has been shown [12] as has enhanced *in vivo* mobilization of fatty acid after injection of somatotropic [11] or feeding of caffeine, a phosphodiesterase inhibitor [16]. The physiological role of the various pituitary factors in the control of lipolysis in this species is unknown but the potential for complex multiple control mechanisms seems possible.

Inhibition of *in vitro* swine adipose tissue lipolysis by insulin is erratic and may be enhanced by the presence of glucose [47], although glucose alone stimulates lipolysis in isolated cells [49]. As for fatty acid synthesis, the marginal *in vitro* insulin response is not substantiated *in vivo* since infusion of insulin decreased plasma fatty acids indicating apparent inhibition of lipolysis [78]. The prostaglandin PGE2 inhibited lipolysis in swine adipose tissue slices [49].

Although the potential endocrine control of adipose tissue metabolism, especially rat adipose tissue, is being unraveled through both *in vitro* and *in vivo* stud-

ies, the mechanisms that function under physiological conditions are less clear. Information regarding the ruminant and swine species is being accumulated, but is far less thorough than for the rat. Since many more studies lie in the future, I would like to make a few cautionary comments.

The use of *in vitro* incubation of adipose tissue is an extremely useful tool by which to make rapid progress in the study of adipose tissue metabolism. However, many factors can influence the results obtained. Isolated adipocytes may give different quantitative or qualitative results than tissue slices; for example, fatty acid synthesis rates are greater in swine adipose tissue than in isolated cells prepared from the same tissue [44] or cells are quantitatively more sensitive to lipolytic hormones than slices but do not respond to prostaglandins as do slices [49]. The incubation conditions can markedly influence the observed result, for example, low pH decreased the lipolytic rate [74], the concentration and source of albumin in lipolytic media greatly influenced the lipolytic rate [17] and glucose, generally inhibitory to lipolytic processes, stimulated swine lipolysis [49]. Negative results obtained with a potential agonist, especially at only one concentration, must be cautiously interpreted; for example, several investigators indicated a negative lipolytic response of rabbit adipose tissue to epinephrine, but recently it has been shown that the antilipolytic α-adrenoceptor activity of epinephrine is more potent than the lipolytic β-adrenoceptor activity [37]. Isoproterenol (a β agonist) stimulated rabbit tissue as did epinephrine in the presence of phentolamine (an α antagonist).

Choice of Animal Used Must Be Cautious

The choice of animals used for a source of adipose tissue must also be made cautiously since the age of the animal, at least partially via cell size differences, will influence the lipogenic [31, 44, 72] or lipolytic [49] rates. The nutritional status of the animal is also important; for example, high fat diets inhibit lipogenesis in swine and bovine adipose tissue [1, 81] as does starvation [36, 57]. Since individual adipose depots each have a distinct pattern of maturation, the observed metabolic effects may be quite different between depots in the same animal [3, 32].

Endocrine agents as well as substrates and cofactors should always be tested over a considerable range of concentrations. The response obtained as well as the dose needed to obtain that response can vary considerably as exemplified by the ontogenic changes in the epinephrine stimulated lipolytic response of swine adipose tissue [46]. The epinephrine-stimulated lipolytic response of swine adipose tissue can also overshoot yielding considerably less than maximal response at high concentrations [unpublished observations]. Certainly effective concentration should not be extrapolated across species; for example, glucose saturates as a lipogenic precursor in rat adipose tissue at about < 5 mM but not in swine tissue until $\geqslant 10$ mM [44], or optimal citrate cleavage enzyme activity in swine adipose tissue should be assayed at quite different substrate concentrations than in rat preparations [48].

The use of *in vivo* experiments circumvents many of the potential hazards of *in vitro* tissue studies. However, *in vivo* studies have their own inherent problems such as the individual animal variation in the observed response is usually large, the observed effects may be secondary effects (for example, via change in blood supply to a tissue or stimulation or suppression of the release of a hormone from an endocrine organ), the nutritional or endocrine status of the animal may not be known and the physical problems of doing *in vivo* experiments with large animals may be extremely great. Well designed *in vivo* experiments constructed with the base of information obtained from *in vitro* approaches and utilizing time course studies along with dose-response curves for the hormone will provide the information needed to understand the endocrine control of adipose tissue and ultimately to control the proliferation of this tissue in the bovine, ovine, or porcine species.

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