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# Immunological manipulation of adiposity

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The deposition of excess energy in the form of triacylglycerol in adipose tissue has become an undesirable trait both in animals and in man. Indeed, it has been the increasingly sedentary lifestyle of man, with the consequential increase in fat accumulation in humans, which ultimately has been the driving force to reduce fat content in domestic species. Since most meats are perceived, rightly or wrongly, as high-fat foods and since saturated animal fats are considered to be the poor relation of their polyunsaturated counterparts found predominantly in vegetable foodstuffs there has been a steady downward trend in the consumption of many meat and dairy products.

In attempting to address this problem endocrinologists enjoyed a 'head-start' since both growth hormone (GH; see Vernon & Flint, 1989) and β-adrenergic agonists (see Hanrahan *et al.* 1986) have long been known to have favourable effects on body composition, increasing lean body mass and decreasing body fat. Certain β-adrenergic agonists behave not only as classical lipolytic agents but also with apparently direct effects on muscle accretion (Bergen *et al.* 1987; Eadara *et al.* 1987; Maltin *et al.* 1987; Yang & McElligott, 1989) whilst GH increases body nitrogen retention, antagonizes the actions of insulin (see Vernon & Flint, 1989) and enhances responsiveness to β-adrenergic compounds (Vernon *et al.* 1987). Despite these favourable effects and despite their ready availability (in recombinant DNA form for bovine and porcine GH) neither of these classes of compounds is licensed for such use in major developed countries. There is genuine concern over the use of β-adrenergic compounds because of problems of short withdrawal periods and possible adverse effects on meat quality (Warris & Kestin, 1988; Warris *et al.* 1989, 1990), whilst the inability to successfully license bovine GH for use in animal production systems continues despite the fact that it must now be the most exhaustively researched (and certainly documented) compound still seeking approval for use and despite the fact that its human counterpart is available for direct injection into humans.

The desire to use non-hormonal approaches, with long withdrawal periods from treatment, led to the search for immunization techniques which could induce similar effects. Not surprisingly these approaches have in the main focused on attempts to manipulate endogenous GH action. The approaches can be broadly defined as immunoneutralization, immuno-enhancement, immuno-mimicry and immunocytotoxicity.

## IMMUNONEUTRALIZATION

The use of antibodies in a classical sense, to bind to and neutralize agents in blood was first attempted, in terms of animal growth, by immunization against somatostatin. This hormone is secreted from the hypothalamus and acts on the pituitary gland to inhibit GH secretion. Thus, by blocking somatostatin action, GH secretion should be increased. Early results in non-improved breeds of sheep were very encouraging (Spencer *et al.*

Table 1. *Effects of growth hormone (GH) deficiency induced with antiserum to rat GH (rGH) on body-weight and fat deposition at 8 weeks of age*†

(Mean values with their standard errors)

| | Body-wt (g) | | Adipocyte cell number ($\times 10^{-6}$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Parametrial (A) | | Subcutaneous (B) | | |
| | Mean | SE | Mean | SE | Mean | SE | B/A |
| Control | 188 | 4 | 21 | 2 | 23 | 3 | 1·1 |
| Anti-rGH: Short term‡ | 145** | 6 | 17 | 2 | 49** | 6 | 2·9 |
| Long term§ | 83** | 3 | 6** | 1 | 14* | 2 | 2·3 |

Mean values were significantly different from control values: *$P<0·05$, **$P<0·01$.

† Neutralization of GH produced a graded decrease in body-weight, and in parametrial cell numbers. By contrast short-term anti-rGH caused a doubling of adipocyte numbers in subcutaneous fat and a much less marked decrease in subcutaneous adipocytes after long-term treatment. This led to an increase in the value for subcutaneous:parametrial adipocytes from approximately 1 up to 2–3.

‡ Anti-rGH administered from 2–25 d of age.

§ Anti-rGH administered from 2–56 d of age.

1983*a*,*b*), but subsequent studies in a number of different species have provided mixed results (see e.g. Trout & Schanbacher, 1990). Despite this fact the approach continues to interest research groups to this very day. One of the surprising elements of this approach was that even when immunization against somatostatin produced improved growth rates it never achieved the reduction in fat content so characteristic of exogenous treatment with GH (Etherton *et al.* 1987; Pell & Bates, 1991).

These surprising findings may be explained in part by the dual characteristics of GH action in adipose tissue since, although GH enhances net mobilization of triacylglycerol from mature adipocytes, paradoxically it appears to stimulate the differentiation of pre-adipocytes in vitro (see Vernon & Flint, 1989). We have recently shown that this latter effect is evident in vivo, since in rats treated neonatally with an antiserum to GH, adipocyte differentiation in internal fat depots is dramatically decreased (Table 1). Surprisingly, however, subcutaneous fat appeared almost totally insensitive to the absence of GH early in life, with sensitivity to GH possibly also varying in this depot depending on age. It is, thus, feasible that neutralization of GH action at critical time-points may be successfully used not just to manipulate total body fat but possibly to induce site-specific effects on adipose tissue.

An alternative strategy which might favour reduced fat deposition involves immunoneutralization of insulin. Direct immunization has, however, proved to be extremely difficult since the pancreas has a considerable capacity to secrete large amounts of insulin until there is sufficient free hormone to elicit biological effects such as maintaining normoglycaemia. An alternative strategy could, however, involve immunoneutralization of gastrointestinal hormones such as gastric inhibitory polypeptide (GIP) which augment insulin secretion, have direct lipogenic effects on adipose tissue and which have been implicated as possible mediators of certain obese syndromes (Ebert & Creutzfeldt, 1987). Even in ruminant species where very little glucose is absorbed from the diet both GIP and glucagon-like peptide-1 appear to have important roles to play in lipid metabolism (Faulkner & Pollock, 1991; McCarthy *et al.* 1992). Since

GIP is secreted in response to dietary triacylglycerol and has direct effects on lipogenesis in adipose tissue (Beck, 1989) neutralization of its actions should prove advantageous in reducing adipose mass. Although immunization against GIP has been shown to reduce insulin responses in rodents (Ebert *et al.* 1979), no long-term consequences on adipose tissue metabolism have been reported.

## IMMUNO-ENHANCEMENT

As its name implies this particular approach involves the process of enhancing hormone action by means of appropriate interactions with antibodies. Thus, rather than neutralizing the actions of GH, antibodies to GH, particularly monoclonal antibodies, have been shown to enhance the biological activity of GH in GH-deficient mice (Aston *et al.* 1986). This approach has been developed in several ways. First, the effect has been demonstrated in domestic livestock utilizing several biological responses to GH. For example, antibodies pre-complexed to GH have been shown to enhance milk production (a GH response) and the diabetogenic effect of GH (Pell *et al.* 1989). The ability of monoclonal antibodies administered alone to enhance the actions of endogenous GH has also been shown (Holder *et al.* 1985). Second, this response is not restricted to monoclonal antibodies since polyclonal antibodies of 'restricted specificity', i.e. raised against a small part of the amino acid sequence of GH, are also effective (Bomford & Aston, 1990). Third, these peptide 'enhancing regions' of GH have been used to actively immunize animals in order to induce the production of antibodies which will complex with and enhance the biological activity of GH (Pell *et al.* 1991).

The precise mechanism of action of such antibodies is still unclear but proposals have included increased half-life, targeting to particular tissues (e.g. liver), interactions with specific subsets of receptors and increased time of receptor occupancy. The fact that GH conjugated to itself has enhanced bioactivity makes this phenomenon even more perplexing (Holder & Aston, 1989).

## IMMUNO-MIMICRY

By utilizing the concept of immune networks in which each antibody produced by the body evokes an anti-antibody, Jerne (1974) proposed that a subset of these anti-antibodies would bind to the same site on the original antibody as the antigen and thereby serve as a so-called internal image of the antigen. We utilized this approach to produce antibody mimics of rat GH and showed them to bind specifically to GH receptors but not prolactin or insulin receptors present in the same tissues. We were further able to show in short-term experiments that such antibodies given to GH-deficient (hypophysectomized) rats could stimulate increased body-weight gain (Gardner *et al.* 1990). These particular studies were too short, however, to address the question of reduced adipose tissue mass. One of the potential spin-offs of antibody mimics of GH is the possibility of producing a number of different antibodies which mimic different parts of the GH molecule. Such a group of antibodies would allow us to address the proposal that GH binds to different subsets of GH receptors (which elicit different biological actions?) via different binding sites on the GH molecule (Thomas *et al.* 1987). Although we were able to produce monoclonal antibodies which mimicked GH in terms of binding

Table 2. *Effects of passive immunization with antisera to pig adipocyte membranes on fat and lean content of pigs*

(Mean values with their standard errors)

| | Back-fat thickness at P2 site (mm) | | Forelimb joint weights (kg) | | | |
|---|---|---|---|---|---|---|
| | | | Fat | | Lean | |
| | Mean | SE | Mean | SE | Mean | SE |
| Control | 12·9 | 0·7 | 1·01 | 0·10 | 2·00 | 0·07 |
| Antibody-treated | 9·0* | 0·7 | 0·76* | 0·11 | 2·23 | 0·14 |

Mean values were significantly different from control values: *$P<0·05$.

to GH receptors, they had very low affinities for the receptor and, thus, did not prove suitable for such studies.

Recently two groups have been examining the interactions of human GH with its receptor utilizing mutagenesis studies (Bass *et al.* 1991; Cunningham *et al.* 1991) and X-ray crystallography (de Vos *et al.* 1992) and have described the precise contact residues involved in this binding interaction. Unexpectedly, their studies revealed that one GH molecule bound to two GH receptors inducing them to dimerize. With knowledge of these interactive sites available to us the prospect of designing anti-receptor antibodies which may serve as hormone agonists becomes a distinct possibility.

## IMMUNOCYTOTOXICITY

Probably the most direct approach to reducing body fat deposition is to consider adipose tissue as an invading organism and produce antibodies capable of binding to and destroying adipocytes. We demonstrated the feasibility of this approach in rats with adipocyte destruction evident immediately post-injection and with very slow recovery of lost adipose mass (Flint *et al.* 1986; Futter & Flint, 1987; Panton *et al.* 1990). This adipocyte destruction identifies this approach as conceptually different from virtually all other approaches to reduce body fat (excepting lipectomy) since it aims to remove cells rather than regulate their metabolism. Rather than becoming lighter than their control counterparts, rats treated with antibodies to adipocytes actually became heavier due to increased protein deposition. Reduced fat deposition has also been achieved in rabbits (Dulor *et al.* 1990) and sheep (Moloney & Allen, 1989; Nassar & Hu, 1991), although without increases in lean body mass. We have, however, shown compensatory increases in lean tissue and significant decreases in adipose tissue content of pigs passively immunized at 6 weeks of age (Table 2). Using an active immunization approach in which pigs were injected with pig adipocyte plasma membranes conjugated to ovalbumin we have demonstrated reduced back-fat thickness in pigs, although without compensatory increases in lean body mass (Table 3).

## SUMMARY

Although hormonal regulators of adiposity are available they as yet have not been licensed for use. Withdrawal periods and delivery systems are still potential problems in

Table 3. *Active immunization of pigs with pig adipocyte plasma membranes*

(Mean values with their standard errors)

| *n* . . . | Control 19 | | Immunized 9 | | Percentage change |
|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | |
| Cold carcass wt (kg) | 69·7 | 1·0 | 67·2 | 2·7 | –3·6 |
| P2 back-fat thickness (mm) | 16·5 | 0·9 | 13·4* | 0·9 | –18·7 |
| Leg muscle wt (kg) | 5·9 | 0·1 | 5·8 | 0·2 | –1·7 |
| Leg subcutaneous fat (kg) | 1·52 | 0·08 | 1·37 | 0·04 | –9·9 |

Mean value was significantly different from control value: $*P<0·05$.

maximizing their effectiveness. Immunization techniques, on the other hand, suffer none of the problems of withdrawal periods or requirement for frequent injection/implantation. As such they are clearly perceived as safe, economic and should have a positive animal welfare image. They are, however, not without their problems. Active immunization in particular involves an autoimmune response and this is typically difficult to evoke and virtually impossible to regulate. In addition, the fact that antibodies may have immunoneutralizing and immunoenhancing properties may explain the apparently contradictory results obtained in various studies as, for example, in the case of immunization against somatostatin. As our knowledge of immune responsiveness and its control increases, however, the possibilities for immune intervention should increase considerably. We may then be faced with ethical rather than practical limitations as to how far we should manipulate growth and body composition.

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