BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **252,** 263–268 (1998)
ARTICLE NO. RC989622

Effects of Antibodies to Adipocytes on Body Weight, Food Intake, and Adipose Tissue Cellularity in Obese Rats

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Received October 1, 1998

Female Wistar rats were fed on a high fat diet for 18 weeks, during which their energy intake increased by 25% and body weight by 50% due to a doubling of adipose tissue tissue stores. Animals were then treated with increasing doses of a sheep polyclonal antiserum to rat adipocytes on days 1-4 and 7 after which they remained untreated for 14 weeks. Antibody treatment reduced body weight by 10% and the weight of parametrial and subcutaneous adipose tissue by 30-40%. This decrease was explicable entirely in terms of a decrease in the number of adipocytes presumably due to adipocyte lysis. These favourable changes in body fat mass were accompanied by improvement in at least one metabolic factor associated with obesity - serum leptin concentrations were significantly reduced in treated animals compared with high fat controls. Genetically obese Zucker rats also showed decreases in the number of adipocytes after treatment with antibodies but in contrast to diet-induced obese rats, they showed a compensatory increase in adipocyte volume which attenuated the effects on body fat mass. These results demonstrate for the first time, the potential to treat diet-induced obesity with antibodies to adipocytes by producing long-term reductions in the number of adipocytes, with minimal side-effects.

Previous studies showed that polyclonal antisera raised against plasma membranes of adipocytes caused adipocyte destruction in rats both *in vitro* (1) and *in vivo* (2-4) and in a variety of species including rabbits (5), sheep (6, 7), pigs (8) and chickens (9). In some studies the lysis of adipocytes was accompanied by increases in lean tissue producing favourable changes in body composition (2, 4). Although all of these findings were obtained using polyclonal antisera, a recent study demonstrated that individual monoclonal antibodies can kill adipocytes in vitro (10). The ability to produce such effects in vivo led to the proposal that this approach might provide a useful therapy for the treatment of obesity. To achieve this human monoclonal antibodies entirely specific for cell surface antigens on adipocytes would be required. They would have to be effective in obese animal models and would probably need to be accompanied by improvement in at least one co-morbidity factor. Previous studies in rats have all been conducted in young, lean, growing animals. In this study we therefore examined both genetically- and diet-induced obese rats, to determine the effects of treatment on food intake and adipose tissue mass and cellularity.

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MATERIALS AND METHODS

Sheep antiserum to rat adipocytes was prepared as previously described (1) except that the immunogen was adipocyte plasma membranes prepared from Zucker rats. A γ-globulin fraction of the serum was prepared using 40% NH_4SO_4 with the exception of study 1, where heat-inactivated serum was used. The γ-globulin was redissolved in, and dialyzed against, 10 mM phosphate buffer pH 7.4 and lyophilized. Prior to use the γ-globulin fraction was dissolved in sterile 0.15M saline at a concentration of 160 mg/ml representing a 4-fold concentration compared with the original antiserum.

Study 1: Effects of antiserum to adipocytes on body weight and composition in fa^+/fa^+ Zucker rats. Zucker rats from the Rowett Research Institute colony were housed in pairs and given ad libitum access to water and food (Labsure irradiated diet). After several weeks animals were treated either with antiserum to adipocytes or non-immune serum in doses of 1, 2, 2 and 4 ml on days 1, 2, 3 and 4 respectively. All doses were given ip - except on day 4 when 2 ml were administered ip and 2×1 ml sc. Treatment was repeated on days 15-18 and again on days 29-32. Food intakes were monitored daily on treatment and twice weekly otherwise. After 5 weeks all animals were killed by cervical dislocation. Adipose tissue depots were removed and weighed, and a portion was removed to prepared isolated adipocytes by collagenase digestion in order to determine mean adipocyte volumes (11). In order to avoid loss of small cells the digestion mixture was examined without washing the cells. Another portion was used to determine dry weight of the tissue in order to determine total adipocyte numbers. Blood was obtained from the

0006-291X/98 $25.00

TABLE 1

Effects of Repeated Treatment with Antiserum to Adipocytes on Body Weight Change and Food Intake in Zucker Rats

	Body weight change (g)		Food intake (g/rat)	
Time (d)	Control	Antiserum	Control	Antiserum
0-5 (treatment)	3.4 ± 1.5	−4.6 ± 1.3**	124 ± 6	72 ± 1**
6-14 (recovery)	19.2 ± 1.8	17.2 ± 5.2	302 ± 1	262 ± 1**
15-19 (treatment)	5.2 ± 3.1	−1.7 ± 1.0	126 ± 6	72 ± 5**
20-28 (recovery)	13.2 ± 2.7	15.0 ± 4.4	320 ± 16	288 ± 9
29-33 (treatment)	11.4 ± 2.1	13.5 ± 5.2	119 ± 2	124 ± 3
0-33	39.7 ± 5.5	39.4 ± 11	990 ± 27	817 ± 12**

Note. Values are means ± SEM for 4 animals per group.
* $p < 0.05$, ** $p < 0.01$ compared with control.

trunk and stored for determination of serum glucose and triglyceride concentrations (Sigma, London) and serum insulin (by RIA).

Study 2: Effect of antiserum to adipocytes on food intake, body weight gain and adiposity in diet-induced obese rats and Zucker (fa^+/fa^+) rats. Mature female Wistar rats approximately 5 months old and weighing approximately 450g were given ad libitum access to standard laboratory chow, milk chocolate and peanuts. After 8 weeks the laboratory chow was replaced with the same chow supplemented with 20% Lard (Special Diet Services, Cheshire, UK). After 18 weeks of this high fat diet the animals were paired for body weight and one of each pair was given sheep antiserum to adipocytes as a γ-globulin fraction, whilst the other received non-immune γ-globulin as a control. The doses, equivalent to 2, 4, 8, 8 and 8 ml/d divided into two equal doses administered at 10.00h and 16.00h on days 1-4 and 7 respectively. Body weights, food and water intakes were monitored daily during treatment and weekly thereafter. Three weeks after treatment both groups of animals were switched to standard laboratory chow (low fat), after a further 2 weeks they were given high fat laboratory chow (high fat), after a further 2 weeks they were given high fat chow, chocolate and nuts (cafe) for 2 weeks and were finally returned to the high fat chow for the last 3 weeks of the study. An additional group were fed on standard laboratory chow throughout to serve as non-obese controls and remained untreated.

Groups of obese Zucker rats weighing approximately 600g were given ad libitum access to standard laboratory chow and water and were also treated with antibodies to adipocytes or non-immune γ-globulin exactly as described above for Wistar rats.

All animals were killed 11 weeks after injection by cervical dislocation and blood obtained from the trunk. Serum was prepared by centrifugation and stored at −20°C until assayed for glucose, triglyceride, insulin and leptin (by ELISA according to manufacturer's instructions, Crystal Chemical Inc., Chicago IL, USA). Major organs were removed, weighed and small portions were preserved for histological analysis in 4% paraformaldehyde. Subcutaneous and parametrial adipose tissue depots were removed, weighed, a small portion used for collagenase digestion to prepare isolated adipocytes and a portion dried to constant weight.

RESULTS

Study 1. Treatment of Zucker rats with antibodies to adipocytes for 5 days led to a decrease in food intake of approximately 40% and a decline in body weight compared with control rats which showed a weight gain (Table 1). Animals treated with antibodies showed an abnormal gait and some loss of locomotor control of the hind limbs for several hours after the first injection and then returned to normal. Subsequent treatments on days 2-5 produced a less marked effect. During the following 9 days off treatment, food intake recovered but was still depressed compared with controls although the rate of body weight gain recovered completely. During the second period of treatment (days 15-19) body weight and food intake were affected to the same extent as in the first period, again with a subsequent recovery towards normal for food intake and again no compensatory increase in body weight gain. In the final period of treatment, however, body weight gain continued to increase and food intake was unaffected, suggesting a complete loss of efficacy of the treatment.

At the end of the treatment period body weight change had not been significantly affected by the treatment. Neither, subcutaneous nor parametrial fat depot weights were significantly affected by treatment with anti adipocyte antibodies (Table 2). The most striking effect of treatment, however, was the large decrease in the number of adipocytes in both depots after treatment, which was compensated for by a large increase (almost 100%) in the mean adipocyte volume. No gross histological changes were evident in any of the major organs of the body nor were there changes in any organ weights due to treatment (results not shown).

Study 2. Female Wistar rats, when given a diet supplemented with chocolate and nuts showed an increase in total energy intake of approximately 50% which then decreased after 2 weeks, remaining elevated by about 25% until week 7 when high fat chow was introduced. Total energy intake again increased to 50% greater than controls and again after a further 2 weeks energy intake decreased, remaining at 20-25% greater than controls fed the low fat chow. Over the 18 weeks pretreatment phase high fat fed rats consumed 2487 kJ/rat/week compared with 1896 kJ/rat/week for the low fat chow. Body weight gain was also increased 3-4 fold compared with chow fed animals (Table 3).

When animals given the high fat diet were treated

TABLE 2

Effects of Antiserum to Adipocytes on Adipose Tissue Weight, Mean Cell Volume, and Cell Number in Obese Zucker Rats

	Adipose tissue dry wt (g)	Mean cell volume (pl)	Adipocyte number ($\times 10^{-6}$)
Parametrial			
Control	26.3 ± 2.2	1027 ± 81	23.7 ± 3.4
Antiserum	24.4 ± 1.3	1960 ± 73***	12.1 ± 0.9**
Subcutaneous			
Control	24.6 ± 1.9	1152 ± 148	25.0 ± 4.3
Antiserum	19.9 ± 1.5	2105 ± 48***	10.4 ± 0.5**

Note. Values are means ± SEM for 4 animals per group.
** $p < 0.01$, ***$p < 0.001$ compared with control.

with antibodies to adipocytes they showed a reduction in food intake and a weight loss whereas animals given non-immune γ-globulin continued to gain weight (Figure 1). Side-effects were evident as described in study 1 although again their severity decreased with repeated treatment despite an escalating dose regime. Body weight continued to decline during the second week after treatment and did not increase during the third week, whereas the controls continued to gain weight throughout the 3-week period. Food intake decreased in rats treated with antibodies during this period (Table 4). During weeks 4 and 5 both high fat groups were switched to low fat chow and showed a surprising reduction in energy intake down to around 55% of the group receiving low fat chow throughout. There was also a concomitant reduction in body weight in both groups (Figure 1). When the animals returned to a high fat chow diet their energy intake recovered and body weight stabilized in both groups and when chocolate and nuts were reintroduced (cafe diet) both groups increased energy intake further and showed body weight gain. For the final weeks of the study the animals were kept on high fat chow and body weight remained stable. Throughout the fluctuating food intakes and body weight changes, the 10% decrease in body weight of antiserum treated animals was maintained when compared with controls, for the 11 weeks post-treatment (Figure 1). Thus during the 11-week post-treatment period the high fat controls gained on average 36g whereas antiserum treated animals lost on average 22g (Table 3). Food intake over the 11 week period was also decreased by approximately 6% in the treated rats although this was achieved entirely within the first 3 weeks (Table 4).

Examination of adipose tissue depots showed that consumption of the high fat diet produced an increase in the weight of these depots of about 80% which was explicable entirely in terms of an increase in the mean cell volume of adipocytes, with no evidence of an increase in cell number (Figures 2-4). Treatment with anti-adipocyte antibodies produced a 40% decrease in body fat depots (Figure 2) which was entirely explained by a reduction in the number of adipocytes (Figure 3) with no effect on mean adipocyte volume (Figure 4). Weights of other major organs of the body were unaffected by treatment (results not shown). Histological analyses revealed occasional abnormalities (haemosiderin in red pulp of spleen, mild tubulo-interstitial nephritis, interstial lymphocyte in filtrations of various tissues) although these could be ascribed to the effects of ageing and they were no more prevalent in treated versus control (high fat) or control (low-fat) groups (results not shown).

The effects of this treatment regime on Zucker rats also induced a transient decrease in food intake and body weight gain but, as in study 1, treated animals

TABLE 3

Effects of ad Libitum Feeding of a High Fat Diet, followed by Treatment with Antibodies to Adipocytes on Body Weight Change in Female Wistar Rats

	Pretreatment body weight (g)			Post-treatment body weight (g)		
Treatment	Week 0	Week 18	Change	Week 0	Week 11	Change
Low fat control	400 ± 13[a]	449 ± 17[a]	49 ± 9[a]	449 ± 17[a]	481 ± 23[a]	32 ± 11[a]
High fat control	387 ± 16[a]	575 ± 38[b]	188 ± 29[b]	575 ± 38[b]	611 ± 39[b]	36 ± 5[a]
High fat and antibodies to adipocytes	386 ± 10[a]	566 ± 47[b]	180 ± 39[b]	566 ± 47[b]	544 ± 40[a]	−22 ± 15[b]

Note. Values are means ± SEM for 7 animals per group. Values with different superscripts in the same column differ ($p < 0.05$).

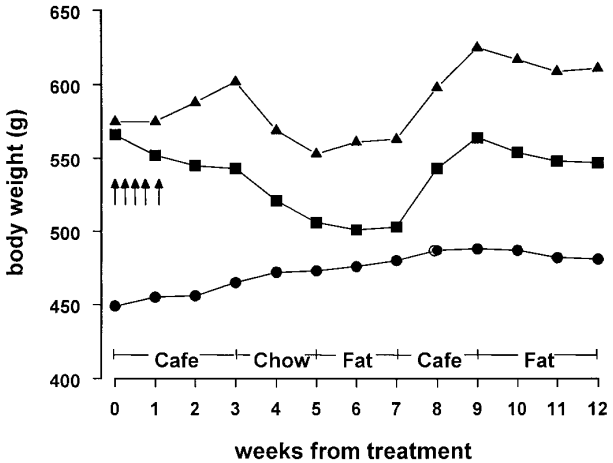

FIG. 1. Changes in body weight in rats on a low fat (chow) diet (circles) or in rats maintained for 18 weeks on a high fat diet then treated with control immunoglobin (triangles) or antibodies to rat adipocyte plasma membranes (squares). Arrows indicate treatment days. Dietary changes are indicated along the x-axis. Chow = low fat; high fat = chow supplemented with 20% fat; cafe = high fat chow plus chocolate and nuts.

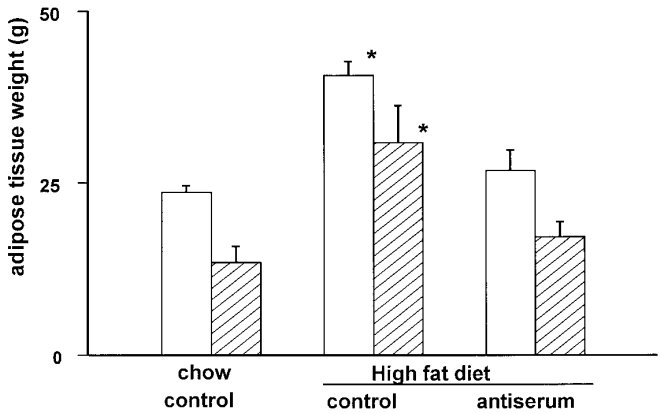

FIG. 2. Wet weights of parametrial (open bars) and subcutaneous (hatched bars) adipose tissue in female Wistar rats fed a low or high fat diet. Values are mean ± SEM for 7 animals per group. *$p<0.05$ compared with chow control.

showed compensatory increases in food intake and body weight gain such that these parameters, as well as adipose tissue weights, were unaffected by treatment (results not shown).

Serum triglyceride concentrations were elevated in Zucker rats but were unaffected by treatment (control 589 ± 61 mg/L, (mean ± SEM) antiserum treated 644 ± 58 mg/L non-obese Wistar control 136 ± 11 mg/L) whereas triglyceride concentrations in rats on high fat chow were not elevated at the end of the study irrespective of treatment (high fat control 141 ± 9, high fat plus antiserum 139 ± 12). Similarly, serum insulin concentrations were increased 17-fold in Zucker rats independent of treatment (Wistar control, 0.9 ± 0.2 ng/ml; Zucker 15.7 ± 4.6 ng/ml) whereas high fat diet induced only a modest increase in basal insulin levels (1.9 ± 0.7 ng/ml) which was not significantly different from chow controls. Glucose concentrations were mildly elevated in Zucker rats irrespective of treatment (1.31 ± 0.09 mg/ml, $p<0.05$) whereas glucose concentration in high fat controls (1.07 ± 0.08 mg/ml) or high fat plus antiserum groups (1.11 ± 0.11 mg/ml) were not significantly different from low fat controls (0.99 ± 0.09 mg/ml). Serum leptin concentrations were increased in high fat control rats (14.9 ± 1.9 ng/ml) compared with chow controls (6.1 ± 1.4 ng/ml, $p<0.01$ Student's t-test). Treatment with antibodies to adipocytes significantly reduced serum leptin concentrations (11.1 ± 2.3 ng/ml, $p<0.01$ compared with high fat controls) although they were still significantly elevated compared with chow fed controls ($p<0.05$).

DISCUSSION

We have previously demonstrated the effectiveness of antibodies to adipocytes in the destruction of adipocytes in vivo with a subsequent improvement in body composition (increased protein decreased fat) in growing animals (1-4, 8). In this study we examined various aspects of treatment which would render this approach feasible for the treatment of obesity. In the first study, involving obese Zucker rats, we demonstrated the typical reduction in food intake and body weight gain during treatment with antibodies to adipocytes which

TABLE 4

Food Intake in Female Wistar Rats after Treatment with Antiserum to Adipocytes or Non-immune Serum

	Energy intake (kJ/rat/period)					
Time (weeks)	0-3	4-5	6-7	8-10	11	Total
Diet	cafe	low fat	high fat	cafe	high fat	
Non-immune serum	6721 ± 683[a]	2362 ± 52[a]	4212 ± 92[a]	8655 ± 1293[a]	1655 ± 27[a]	23609 ± 1613[a]
Antiserum	4934 ± 191[b]	2452 ± 105[a]	4372 ± 150[a]	8639 ± 1200[a]	1743 ± 209[a]	22171 ± 766[a]
Low fat control (low fat chow throughout)	6074 ± 30[a]	4184 ± 71[b]	4047 ± 89[a]	6249 ± 168[a]	1790 ± 15[a]	22344 ± 77[a]

Note. Values are means ± SEM. Values with different superscripts differ ($p < 0.05$).

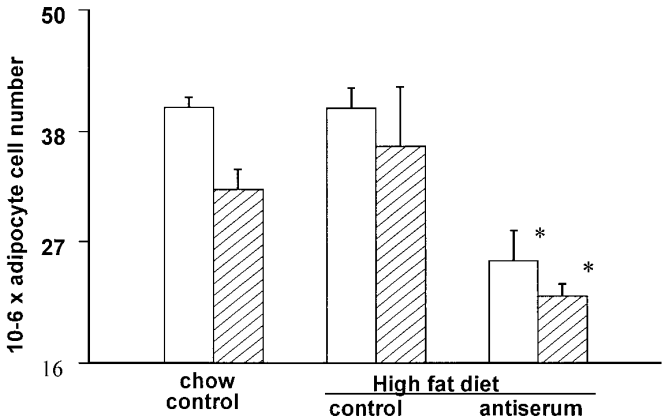

FIG. 3. Adipocyte numbers in female Wistar rats fed a low or high fat diet. Values are mean ± SEM for 7 animals per group. *p<0.05 compared with chow control.

we had previously shown in young, non-obese rats. In addition we showed that this effect could be reproduced two weeks later. This was in contrast to a previous study where we showed that a single injection given two weeks after the initial treatment was ineffective (see 12), due to neutralization of the injected antibody by the rat anti-sheep IgG response. Our success in the present study may have been due to the repeated dosing effectively overcoming the neutralizing antibodies. Even so, when a third treatment period was attempted, there was a loss of efficacy presumably due to an increased effectiveness of the response of the rats to the injected immunoglobulin. Clearly this anti-sheep IgG response is irrelevant in a clinical context, where homologous antibodies would be used, but it serves to demonstrate one of the limitations of the current model.

Treatment of Zucker rats was very effective in decreasing the number of adipocytes in two adipose tissue depots although there was no significant effect on adipose tissue mass due to a compensatory increase in the mean adipocyte volume. To explore whether this phenomenon was peculiar to the Zucker rats, we induced obesity in normal Wistar rats using a high fat chow supplemented with chocolate and nuts. The initial effects of treatment were similar to Zucker rats, with a 10% reduction in body weight gain and a 50% reduction in food intake during the week of treatment. After 3 weeks, during which time appetite remained suppressed by 10-15%, we superimposed dietary changes, typical of those which might be anticipated in treating obesity, ie a switch to a low fat diet. We were surprised to see that both control and antibody-treated animals markedly suppressed food intake, well below animals which were on a low fat chow throughout the study. Subsequent changes in diet led to parallel changes in food consumption and body weight gain in treated and control rats, resulting in the maintenance of a 10% reduction in body weight in treated animals compared with controls, 12 weeks after treatment. In contrast with the Zucker rats, this loss of weight represented a large decrease in adipose tissue mass, which was due solely to a loss of adipocytes, and with no evidence of any compensatory mechanism in terms of increased storage of triglyceride in surviving adipocytes. This illustrates a major difference between genetic- and diet-induced obesity models and questions the usefulness of the Zucker rat for studies of this nature. The differences in these models were highlighted by the huge increase in serum insulin in Zucker rats which was much more modest in diet-induced obese rats as well as the greatly elevated serum triglyceride levels in Zucker rats which were not apparent in animals on a high fat diet.

This study clearly demonstrates for the first time the ability of passive immunization to produce significant reductions in adipose tissue mass in obese animals and to maintain these effects for several months without further treatment. In addition serum leptin concentrations were significantly reduced by treatment with antibodies to adipocytes. This suggests that improvements in co-morbidity factors may well accompany treatment with antibodies to adipocytes although clearly more detailed studies are required to assess this fully.

Treatment did produce immediate side-effects and whether these are due to antibodies which are specific to adipocytes or due to cross-reacting antibodies in the polyclonal antiserum is unclear at the moment. However, there were no long-term side effects in terms of animal behaviour, or in terms of the histology of major organs. In addition the transient effects on behaviour appeared to be self-limiting since they became less apparent upon retreatment, even with an escalating

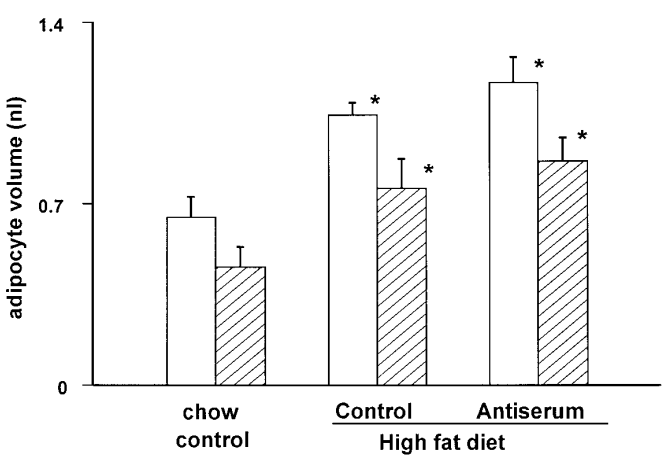

FIG. 4. Mean adipocyte volumes in female Wistar rats fed a low or high fat diet. Values are mean ± SEM for 7 animals per group. *p<0.05 compared with chow control.

dose. One possible explanation for this is that treatment leads to complement activation, and thereby depletion, which thus limits the cytotoxicity of the antibodies. In support of this proposal a previous study of ours, with relatively low doses of antiserum, showed that serum complement falls to low levels within 12h of treatment before recovering at 24h (4).

Finally, in these studies, although we have demonstrated effects with polyclonal antisera there is reason to suppose that single, monoclonal antibodies might be ineffective because of their inability to fix complement efficiently. Recent studies (10, 13) have, however, shown that individual monoclonal antibodies produced against porcine adipocytes will kill rat or porcine adipocytes in vitro. Pairs of antibodies could successfully reduce body fat content in vivo in rats (10) and a single monoclonal antibody was shown to be effective in reducing fat deposition in pigs (13). Thus, if human monoclonal antibodies can be identified with appropriate specificity and complement-fixation properties, the goal of an anti-obesity immunization therapy will be even closer.

ACKNOWLEDGMENTS

The author thanks Miss M. Gardner for most of the analyses, Professor P. Mathieson and Dr. S. Thiru for histological analyses, Dr. Chen Bing for leptin analyses, and Mrs. M. Knight for the preparation of the manuscript. This work was funded by ObeSys Ltd whose support we gratefully acknowledge.

REFERENCES

1. Flint, D. J., Coggrave, H., Futter, C. E., Gardner, M. J., and Clarke, T. J. (1986) *International Journal of Obesity* **10,** 69–77.
2. Futter, C. E., and Flint, D. J. (1987) *In* Recent Advances in Obesity Research: V. (Berry, E. M., Blondheim, S. H., Eliahou, H. E., and Shafrir, E., Eds.), pp 181–185. John Libbey and Company, London.
3. Futter, C. E., Panton, D., Kestin, S., and Flint, D. J. (1992) *International Journal of Obesity* **16,** 615–622.
4. Panton, D., Futter, C., Kestin, S., and Flint, D. J. (1990) *American Journal of Physiology* **258,** E985–E989.
5. Dulor, J. P., Reyne, Y., and Nougues, J. (1990) *Reproduction, Nutrition and Development* **30,** 49–58.
6. Moloney, A. P., and Allen, P. (1989) *Proceedings of the Nutrition Society* **48,** 14A.
7. Nassar, A. H., and Hu, C. Y. (1991) *Journal of Animal Science* **69,** 578–586.
8. Kestin, S., Kennedy, R., Tonner, E., Kiernan, M., Cryer, A., Griffin, H., Butterwith, S., Rhind, S., and Flint, D. J. (1993) *Journal of Animal Science* **71,** 1486–1494.
9. Butterwith, S. C., Kestin, S., Griffin, H. D., Beattie, J., and Flint, D. J. (1992) *Comparative Biochemistry and Physiology* **101B,** 147–151.
10. Wright, J. T., and Hausman, G. J. (1995) *Obesity Research* **3,** 265–272.
11. Vernon, R. G. (1977) *Biol. Neonate* **32,** 15–23.
12. Flint, D. J. (1990) *In* Biotechnology and Genetic Engineering Reviews, Volume 8 (M. P. Tombs, Ed.), pp 97–131. Intercept, Andover.
13. De Clerq, L., Mourot, J., Genart, C., Davidts, V., Boone, C., and Remacle, C. (1997) *Journal of Animal Science* **75,** 1791–1797.