INCREASED LIPOGENESIS IN BROWN ADIPOSE TISSUE OF LACTATING RATS FED A CAFETERIA DIET

The possible involvement of insulin in brown adipose tissue hypertrophy

Loranne AGIUS, Barbara J. ROLLS*, Edward A. ROWE* and Dermot H. WILLIAMSON

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE and **Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, England*

Received 21 October 1980; revised version received 19 November 1980

Rats fed a cafeteria diet have increased rates of brown adipose tissue lipogenesis (concomitant with decreased rates of mammary gland lipogenesis) but there was no significant brown adipose tissue hypertrophy, in contrast to the hypertrophy that occurs in non-lactating rats. Insulin treatment and the cafeteria diet together had a synergistic effect on the weight of brown adipose tissue in lactating rats. This suggests that insulin may be involved in brown adipose tissue hypertrophy and a higher insulin level than normally observed during lactation may be required for hypertrophy to occur.

1. Introduction

Brown adipose tissue hypertrophies in rats that become hyperphagic when allowed access to a palatable, high-energy (cafeteria) diet, and this hypertrophy may be associated with increased dietary-induced thermogenesis [1–3]. It also hypertrophies during the physiological hyperphagia of pregnancy but regresses during lactation, when the food-intake exceeds that of pregnancy [4]. Cafeteria feeding during lactation in the rat results in voluntary overeating, poor litter growths [5] and impaired mammary gland lipogenesis [6]. Here we have investigated the effects of cafeteria-feeding on brown adipose tissue of lactating rats. The hypertrophy of brown adipose tissue during pregnancy and its regression during lactation may be related to the high and low insulin levels, respectively, of these two states [4]. To investigate the possibility that brown adipose tissue weight changes may be related to the plasma insulin level we examined the effects of insulin treatment on brown adipose tissue of lactating rats that were fed the chow diet or the cafeteria diet.

Brown adipose tissue also hypertrophies during prolonged cold exposure when it is important in non-shivering thermogenesis and the rate of lipogenesis in vivo measured by the incorporation of ^3H_2O into lipid also increases [7–9]. This increase in lipogenesis may be related to the increase in thermogenesis [8,9]. Here, the effects of the cafeteria-diet on brown adipose tissue lipogenesis in lactating rats were assessed.

The results show that lactating rats fed the cafeteria diet have increased rates of brown adipose tissue lipogenesis (concomitant with decreased rates of mammary gland lipogenesis) but there was no significant brown adipose tissue hypertrophy, in contrast to the hypertrophy that occurs in non-lactating rats. Insulin treatment and the cafeteria diet together had a synergistic effect on the weight of brown adipose tissue in lactating rats. This suggests that insulin may be involved in brown adipose tissue hypertrophy and a higher insulin level than normally observed during lactation may be required for hypertrophy to occur.

2. Materials and methods

The rats were fed ad libitum on a standard chow diet (Dixons FFG(M), Aldenham Park, Elstree, Herts) and were subject to a 12 h light:12 h dark cycle, the light period running from 08:00–20:00 h. The body weight of the rats at mating was 175–250 g. Rates of lipogenesis in vivo were measured in lactating rats 10–12 days post-partum by the incorporation of ^3H_2O into lipid as in [4], these experiments were started between 09:00 and 10:00 h.

Experiment 1: To investigate the effects of a cafeteria diet and insulin treatment on brown adipose tissue hypertrophy and lipogenesis

Rats of the Hooded Lister strain were used. At birth the litters were culled to 8 pups and the lactating rats were divided into two groups: one was left on chow ad libitum and the other was given the cafeteria diet

in addition to chow. This consisted of cheese crackers, chocolate chip cookies and potato crisps (for composition of foods see [10]). Half of the lactating rats in each group were given daily insulin injections s.c. (1 unit in 0.2 ml, Isophane Insulin Injection, Nordisk Insulinlaboratorium, Copenhagen), the rest were given daily saline injections s.c. (0.2 ml, 0.15 M NaCl). The injections were given from the day after parturition for 10–12 days. The last injection was given 60 min before the experiment. Rates of lipogenesis in vivo were determined as above. Virgin rats were fed on chow or the cafeteria diet for 7 days for comparison.

Experiment 2: To investigate the effects of a cafeteria diet and insulin deficiency on brown adipose tissue in a different strain of rats

Virgin rats of the Wistar and Lister strain respond differently to a cafeteria diet by the degree of voluntary overeating (B. J. R., E. A. R., unpublished). It was therefore of interest to investigate the effects of the cafeteria diet on lactating rats of the Wistar strain. The Wistar lactating rats were culled to 10 pups at birth. One group of lactating rats was left on chow and another group was given the cafeteria diet in addition to chow (comprising cheese crackers and chocolate chip cookies). Insulin deficiency was induced in a third group of lactating rats (fed chow) with streptozotocin [11] (Upjohn Co., Kalamazoo, MI); a single injection i.p. (45 mg/kg body wt, 4.5% solution in 0.01 M citrate buffer (pH 4.5)) was given the day after parturition. These rats were glycosuric and polyuric throughout lactation, and the mean blood glucose level at the end of the experiment was 18 mM.

3. Results

3.1. *Brown adipose tissue hypertrophy in virgin and lactating rats*

Hooded Lister virgin rats have more ($P < 0.0005$) interscapular brown adipose tissue than Albino Wistar virgin rats (table 1). The cafeteria diet resulted in hypertrophy of the tissue in virgin rats of both strains; the tissue wt/body wt ratio was increased in the Wistar

Table 1
Effects of cafeteria feeding and insulin treatment on brown adipose tissue hypertrophy in virgin and lactating rats

Experiment	Diet	Body wt (g)	Interscapular brown adipose tissue		
			Wet wt (mg)	% fat	Wet wt (%) Body wt
1. Hooded Lister rats					
Virgin	Chow (8)	227 ± 10	559 ± 52	52.8 ± 5.1	0.245 ± 0.016
Virgin	Cafeteria (8)	231 ± 5	770 ± 59[a]	59.1 ± 2.8	0.331 ± 0.021[b]
Lactating saline-injected	Chow (5)	268 ± 9	446 ± 25	60.4 ± 1.7	0.168 ± 0.011[b]
Lactating saline-injected	Cafeteria (6)	254 ± 16	474 ± 38	48.8 ± 3.0	0.200 ± 0.012[a,c]
Lactating insulin-injected	Chow (4)	253 ± 6	462 ± 65	49.3 ± 3.6	0.183 ± 0.024[a]
Lactating insulin-injected	Cafeteria (6)	263 ± 8	683 ± 38[d]	53.6 ± 3.1	0.262 ± 0.017[d]
2. Albino Wistar rats					
Virgin	Chow (8)	205 ± 2	284 ± 16	46.3 ± 2.3	0.138 ± 0.007
Virgin	Cafeteria (6)	214 ± 3[a]	450 ± 39[b]	50.9 ± 2.4	0.210 ± 0.016[b]
Lactating untreated	Chow (6)	343 ± 11	277 ± 21	42.8 ± 1.6	0.082 ± 0.007[b]
Lactating untreated	Cafeteria (7)	329 ± 11	302 ± 23	39.5 ± 3.2	0.096 ± 0.008[b]
Lactating diabetic	Chow (5)	282 ± 4[d]	145 ± 16[d]	22.0 ± 4.3[d]	0.051 ± 0.005[d]

Values that are significantly different by the Student's t-test from those of the corresponding chow-fed virgin rat are shown: [a]$P < 0.05$; [b]$P < 0.005$ and from the corresponding chow-fed lactating rat are shown: [c]$P < 0.05$; [d]$P < 0.005$

For experimental details see text. Values are means ± SE with no. rats in parentheses

strain ($P < 0.005$; 52% increase) and in the Lister strain ($P < 0.005$; 35% increase). Lactating rats have less interscapular brown adipose tissue on a body weight basis than virgin rats (31% decrease in the Lister strain, 41% decrease in the Wistar strain). The cafeteria diet had little effect on brown adipose tissue weight in untreated lactating rats in contrast to the hypertrophy induced in virgin rats. Daily insulin treatment had no significant effect on brown adipose tissue weight in lactating rats fed chow (table 1). However, insulin treatment and the cafeteria diet together significantly increased brown adipose tissue weight ($P < 0.005$; 56% increase) compared to chow-fed saline-injected rats. The increase in tissue wt/body wt ratio was significant relative to both the insulin-treated chow-fed rats ($P < 0.01$) and the untreated cafeteria-fed rats ($P < 0.01$). It is noteworthy that although the energy intake was higher (14%) in the untreated cafeteria-fed group relative to the untreated chow-fed group, daily insulin treatment did not affect the energy intake in either the chow- or the cafeteria-fed rats (not shown). Insulin deficiency induced with streptozotocin in lactating rats resulted in a decrease in weight of interscapular brown adipose tissue (table 1).

3.2. *Rates of lipogenesis in lactating rats*

The cafeteria diet increased brown adipose tissue lipogenesis (Lister rats, 7.7-fold; Wistar rats, 5.2-fold) but inhibited mammary gland lipogenesis (Lister rats, 82%; Wistar rats, 61%). However, it had no effect on hepatic and white adipose tissue lipogenesis (table 2).

Insulin deficiency decreased the rate of lipogenesis in the 4 tissues studied. Daily insulin treatment of rats fed chow increased lipogenesis in liver and brown and white adipose tissue but not in mammary gland; these rates were the same as in lactating rats given a single insulin injection before the experiment (not shown) indicating that long-term insulin treatment had no additional effect on rates of lipogenesis. Insulin treatment of rats fed the cafeteria diet increased mammary gland lipogenesis 4-fold ($P < 0.0005$) but the rate was 36% lower ($P < 0.01$) than in the insulin-treated lactating rats fed chow. A similar response of mammary gland lipogenic rate in vivo to insulin is observed after short-term inhibition of mammary gland lipogenesis induced with an oral load of triacylglycerol [12]. In the insulin-treated cafeteria-fed rat hepatic and white adipose tissue lipogenesis were the same as in the insulin-treated chow-fed rat but the rate of brown adipose tissue lipogenesis was 2-fold higher ($P < 0.01$) (table 2).

4. Discussion

It has been reported that brown adipose tissue hypertrophies in rats fed on a cafeteria diet, and this is accompanied by an increase in thermogenesis [1,3]. Two points emerge from the present study:

(i) The cafeteria diet did not increase brown adipose tissue weight in lactating rats, however significant hypertrophy occurred in cafeteria-fed lactating rats given daily insulin injections;

Table 2
Effects of cafeteria feeding and insulin treatment on rates of lipogenesis in vivo in lactating rats

Experiment	Diet		Mammary gland	Brown adipose tissue	White adipose tissue	Liver
1. Hooded Lister rats						
Saline-injected	Chow	(5)	89.0 ± 8.2	7.1 ± 1.3	1.0 ± 0.1	18.6 ± 3.0
Saline-injected	Cafeteria	(6)	15.7 ± 6.1[b]	54.5 ± 10.1[b]	1.3 ± 0.3	18.0 ± 2.9
Insulin-injected	Chow	(4)	100.8 ± 9.0	49.2 ± 8.8[b]	5.8 ± 2.1[a]	58.0 ± 2.8[b]
Insulin-injected	Cafeteria	(6)	61.7 ± 7.8[a]	83.5 ± 8.2[b]	6.1 ± 1.5[a]	52.0 ± 4.6[b]
2. Albino Wistar rats						
Untreated	Chow	(6)	104.0 ± 3.9	5.1 ± 1.0	2.3 ± 0.6	14.7 ± 1.3
Untreated	Cafeteria	(7)	40.6 ± 7.4[b]	26.3 ± 7.8[a]	1.2 ± 0.3	16.7 ± 2.7
Diabetic	Chow	(3)	16.0 ± 2.9[b]	1.8 ± 0.3[a]	0.8 ± 0.1[a]	6.6 ± 0.9[b]

Values that are significantly different by the Student's t-test from the corresponding values of the saline-injected (Lister) or untreated (Wistar) chow-fed rats are shown: [a] $P < 0.05$; [b] $P < 0.005$

For experimental details see text. Rates of lipogenesis are expressed as μmol ^3H_2O incorporated into lipid . h^{-1} . g wet wt^{-1}. The values are means ± SE with no. rats in parentheses

(ii) Lactating rats fed the cafeteria diet had increased rates of brown adipose tissue lipogenesis.

After parturition in the lactating rat there is a fall in plasma insulin concentration [13] and a decline in brown adipose tisue weight [4]. Here, lactating rats made diabetic after parturition showed a further decline in brown adipose tissue weight suggesting that the regression of the tissue may be related to the decrease in plasma insulin concentration. While insulin treatment and the cafeteria diet separately had no significant effect on brown adipose tissue weight, together they had a synergistic effect in lactating rats. This suggests that an elevated insulin concentration may be necessary for the cafeteria diet to induce brown adipose tissue hypertrophy. It is not known what hormonal changes are involved in the hypertrophy of brown adipose tissue induced by the cafeteria diet in non-lactating rats. Noradrenaline treatment has been shown to result in brown adipose tissue hypertrophy [14,15] and the possibility that the insulin treatment in this study affected the noradrenaline levels cannot be excluded. It is noteworthy, however, that male rats fed on the cafeteria diet became hyperinsulinaemic [10].

The cafeteria diet had two effects on brown adipose tissue lipogenesis in lactating rats:

(i) An increase in lipogenesis compared to both lactating rats fed chow (by 5–7-fold) and virgin rats fed the cafeteria diet (by 2–3-fold; not shown);

(ii) An increased lipogenic capacity after insulin treatment that was 2-fold higher than in the insulin-treated lactating rat fed chow (table 2; [16]).

It is noteworthy that this increased lipogenic rate in the insulin-treated cafeteria-fed lactating rats was similar to that in insulin-treated virgin rats fed chow [16] indicating that the cafeteria diet prevents the decrease in lipogenic capacity of brown adipose tissue that occurs during normal lactation [16].

Brown adipose tissue lipogenesis in vivo increases several-fold after a glucose load in virgin rats but not in lactating rats [4]. It is of interest therefore that cafeteria feeding increased brown adipose tissue lipogenesis in the lactating rat. The low rate of lipogenesis in the lactating rat fed ad libitum or given a glucose load may be important in conserving lipogenic substrates for the mammary gland [4]. This raises the question whether the increase in brown adipose tissue lipogenesis in the lactating rat fed the cafeteria diet may be related to the decrease in mammary gland lipogenesis. The high prolactin concentration and low insulin level during normal lactation are possibly both involved in the direction of lipogenic substrates to the mammary gland [17]. Thus the decreased partitioning of lipogenic substrates to the gland and increase to brown adipose tissue in the cafeteria fed lactating rat may be related to alterations in endocrine state.

Although cafeteria feeding resulted in an increase in brown adipose tissue lipogenesis in the lactating rat, it did not produce the same degree of hypertrophy as in the virgin rat and we suggest that a higher circulating insulin concentration may be required for the diet to induce brown adipose tissue hypertrophy.

Acknowledgements

This work was supported by the Medical Research Council and the US Public Health Service (grant no. AM-11748). L. A. is a Commonwealth Scholar. D. H. W. is a member of the External Staff of the Medical Research Council (UK).

References

[1] Rothwell, N. J. and Stock, M. J. (1979) Nature 281, 31–35.
[2] Himms-Hagen, J. (1979) Can. Med. Assoc. J. 121, 1361–1364.
[3] Brooks, S. L., Rothwell, N. J., Stock, M. J., Goodbody, A. E. and Trayhurn, P. (1980) Nature 286, 274–276.
[4] Agius, L. and Williamson, D. H. (1980) Biochem. J. 190, 477–480.
[5] Rolls, B. J., Rowe, E. A., Fahrbach, S. E., Agius, L. and Williamson, D. H. (1980) Proc. Nutr. Soc. 39, 51A.
[6] Agius, L., Rolls, B. J., Rowe, E. A. and Williamson, D. H. (1980) Biochem. J. 186, 1005–1008.
[7] McCormack, J. G. and Denton, R. M. (1977) Biochem. J. 166, 627–630.
[8] Trayhurn, P. (1979) FEBS Lett. 104, 13–16.
[9] Rath, E. A., Salmon, D. M. W. and Hems, D. A. (1979) FEBS Lett. 108, 33–36.
[10] Rolls, B. J., Rowe, E. A. and Turner, R. C. (1980) J. Physiol. (London) 298, 415–427.
[11] Schein, P. S., Alberti, K. G. M. M. and Williamson, D. H. (1971) Endocrinology 89, 827–834.
[12] Agius, L. and Williamson, D. H. (1980) Biochem. J. 192, 361–364.
[13] Flint, D. J., Sinnett-Smith, P. A., Clegg, R. A. and Vernon, R. G. (1979) Biochem. J. 182, 421–427.
[14] Leblanc, J. and Villemaire, A. (1970) Am. J. Physiol. 218, 1742–1745.
[15] Desautels, M. and Himms-Hagen, J. (1979) Can. J. Biochem. 57, 968–976.
[16] Agius, L. and Williamson, D. H. (1980) Biochem. Soc. Trans. 8, 598–599.
[17] Agius, L., Robinson, A. M., Girard, J. R. and Williamson, D. H. (1979) Biochem. J. 180, 689–692.