# EFFECTS OF VASOPRESSIN ON LIPOGENESIS IN OBESE MICE

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## 1. Introduction

Vasopressin is one of several catabolic hormones which have been shown to inhibit fatty acid synthesis in lean mice [1]. Using the perfused liver preparation, it can be shown that the inhibitory action of vasopressin is rapid in onset suggesting that vasopressin exerts a primary inhibitory effect on lipogenesis which probably occurs at the acetyl CoA carboxylase locus [2]. Livers of genetically obese (ob/ob) mice exhibit resistance to the inhibitory effects of vasopressin, adrenalin and angiotension II on fatty acid synthesis although these hormones still stimulate glycogenolysis [3–5]. Such an impairment in inhibition of fatty acid synthesis might be expected to predispose towards obesity. Since the resistance to vasopressin's action on the liver persists even after severe dietary restriction in the ob/ob [3], it has been suggested that this defect may closely reflect the inborn lesion in obese mice [5].

To study further whether resistance to catabolic hormones is a result of the obesity itself or a primary lesion of genetic origin, we have determined the resistance to the inhibitory action of vasopressin on lipogenesis in the perfused livers of mice made obese by treatment with gold thioglucose or by feeding a high fat diet. Furthermore, we have examined the effect of vasopressin in the genetically obese db/db mice. These latter mice exhibit similar biochemical lesions to ob/ob mice, although the genetic lesion is located on chromosome 4 rather than chromosome 6 [6].

## 2. Materials and methods

Obesity was induced in CFLP female mice (22–24 g body wt) (Anglia Lab, Alconbury, Hunts) either by a single intraperitoneal injection of gold thioglucose, (0.9 mg/g body wt); kindly supplied by Schering Corp., or by feeding on a high protein–high fat diet. The control mice and those given gold thioglucose were maintained on Oxoid rat and mouse breeders diet (H.C. Styles, Bewdley, Worcs) ad libitum. The high protein–high fat diet had the composition (%): butter 38.9; barley 4.1; maize meal 1.3; Sussex oats 3.4; bran 3.4; white fish meal 4.2; yeast 1.3; meat and bone 8.4; dried skim milk 31.8; NaCl 0.4; $NaH_2PO_6 \cdot 2\,H_2O$ 0.05; vitamin mixture 2.6 [7]; and was additionally supplemented with biotin (0.1 mg/kg), D-α-tocopheryl acetate (300 mg/kg) and selenite (7 mg/kg).

The gold thioglucose-treated mice were taken for the perfusion studies 7 weeks after injection by which time they were 50.7 ± 3.0 g body wt. The high protein–high fat diet-treated mice were fed on the diet for 22 weeks by which time they were 53.4 ± 2.9 g body wt. The control CFLP mice were 30–35 g body wt.

The db/db mice were bred in these laboratories and originated from a stock maintained at Jackson Lab., (Bar Harbor, ME). The genetic background of these mice was C57BL/KS and the + allele was linked with the coat-colour allele (misty (m)). At the time of carrying out the perfusions the mice were aged between 10–14 weeks. C57BL/6J ob/ob mice were obtained from Olac, Bicester.

Livers from fed mice were perfused between 10:00 h and 14:00 h at 37°C with a perfusate composed of Krebs-Ringer bicarbonate buffer, bovine serum albumin (2%, w/v), washed aged human erythrocytes, glucose, (15 mM) and lactate (10 mM). The perfusate was recirculated for 50 min before $^3H_2O$ and vasopressin (25 nM) were added. Non-recirculating perfusion was

† Deceased

continued for a further 40–50 min. Fatty acid synthesis was determined as in [6].

Studies in intact mice involved intraperitoneal injection of $^3H_2O$ and subcutaneous injection of vasopressin (100 mU/mouse), while control mice received saline. After 1 h a small blood sample was taken to measure the specific radioactivity of $^3H_2O$, liver samples were removed quickly and dropped into liquid nitrogen. The meal fed mice were offered food from 10:00–14:00 h daily and had been adapted to this procedure for 28 days prior to the experiment.

## 3. Results and discussion

In the perfused livers from freely-fed, gold thioglucose-treated mice there was a significant increase ($P < 0.05$) in the rate of fatty acid synthesis relative to that found in age-matched lean mice (table 1). This agrees with earlier studies in vivo [8]. The rate of fatty acid synthesis in perfused livers from mice fed the high butter fat diet did not significantly differ from the rate in the control mice. In all 3 groups of mice, vasopressin (25 nM) reduced the rate of lipogenesis by ~50%.

Earlier studies [3] showed that in genetically obese (ob/ob) mice of the Imperial College strain [9], there was a complete failure of vasopressin to exert its normal inhibitory effect. These mice often attain >80 g body wt of which >60% is fat. Such mice show many metabolic derangements and it has proved difficult to delineate which of these derangements are primary and which arise as a secondary consequence of the obese condition. One approach to this problem has been to severely reduce the food intake of ob/ob mice. In such mice vasopressin [3], adrenalin and angiotension II [5] all failed to inhibit lipogenesis. However, although the body weight of these diet-restricted mice can be maintained at a similar level to that of control mice homozygous for the normal allele, such mice still contain significantly more fat than controls.

Treatment of mice with gold thioglucose or the feeding of the high protein–high fat diet both lead to massive obesity (fat content of the carcass >40%). Thus the finding that the livers of such mice respond normally to the inhibitory effects of vasopressin on lipogenesis does not support the notion that resistance to the action of vasopressin is the result of the obese state. Furthermore, the finding that db/db mice also respond normally to the inhibitory effect of vasopressin on lipogenesis (table 1) gives further evidence to the view that resistance to catabolic effects of hormones on the liver of ob/ob mice may closely reflect the inborn error [5].

The metabolic consequences of the possession of both the ob/ob and db/db genes are dependent on the genetic background (C57BL/6J or KSJ) of the mice [10,11]. Thus, both pairs of alleles when placed on the same background produce an identical metabolic pattern. The obese (ob/ob) mice used in earlier studies in which resistance to vasopressin was demonstrated came from a mixed outbred stock into which the ob/ob gene was introduced. To ensure that resistance to the inhibitory effect of vasopressin on fatty acid synthesis is not merely the result of the particular phenotypic background of Imperial College (ob/ob) mice, studies have now been carried out in C57BL/6J ob/ob mice (table 2). Again, resistance to the normal inhibitory action was demonstrated.

Table 1
Effect of vasopressin on lipogenesis in perfused mouse liver

| Source of liver | Fatty acid synthesis (μmol C2 units . $h^{-1}$ . g fresh tissue$^{-1}$) | |
|---|---|---|
| | No addition | Vasopressin (25 nM) |
| Lean mice | 13.0 ± 1.6 (16) | 7.8 ± 1.7[a] (8) |
| Gold thioglucose-treated mice | 20.0 ± 2.7 (4)[b] | 9.0 ± 1.0[a] (3) |
| High protein–high fat-fed mice | 9.7 ± 0.7 (4) | 4.7 ± 0.9[a] (5) |
| db/db Mice | 20.6 ± 3.8 (4) | 6.1 ± 0.9[a] (4) |

[a] Significantly different from controls $p < 0.05$; [b] Significantly different from lean mice $p < 0.05$

Table 2
Effect of vasopressin on lipogenesis in intact mice

| Mice | Time of day (h) | Fatty acid synthesis (μmol C2 units . $h^{-1}$ . g liver$^{-1}$) | |
|---|---|---|---|
| | | Control | Vasopressin-treated |
| C57BL/6J +/? | 06:00 | 33 ± 3 (9) | 17 ± 4 (8)[a] |
| +/? | 09:00 | 22 ± 4 (4) | 10 ± 1 (3)[b] |
| C57BL/6J | 06:00 | 81 ± 17 (3) | 73 ± 15 (3) |
| ob/ob | 09:00 | 61 ± 12 (4) | 61 ± 8 (4) |
| Meal-fed CFLP | 12:00 | 93 ± 6 (7) | 69 ± 5 (7)[a] |

[a] Significantly different from controls, $p < 0.01$; [b] Significantly different from controls, $p < 0.05$

The rates of hepatic lipogenesis in vivo in ob/ob mice is generally much faster than the rates of lipogenesis in lean mice, gold thioglucose or dietary-induced obese mice. Resistance to vasopressin could therefore be a consequence of these higher rates of lipogenesis. High rates of lipogenesis can be achieved in normal mice that have been adapted to a meal-feeding regime [12,13]. In our experiments (table 2) such mice had similar rates of lipogenesis to the genetically ob/ob mice, but in the meal-fed mice vasopressin exerted its normal inhibitory effect. All of these studies add support to the view that the failure of vasopressin to inhibit lipogenesis in ob/ob mice is directly linked to the possession of the ob/ob allele.

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