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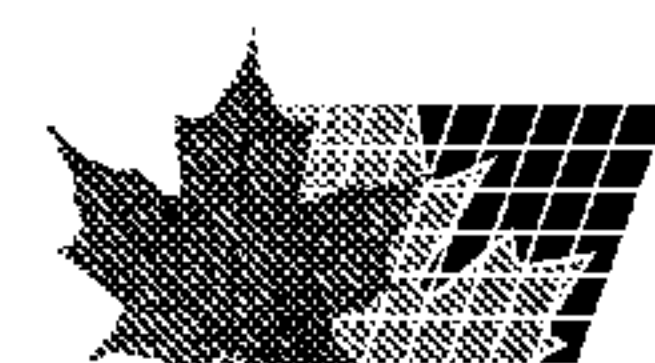
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(54) Title: MODIFICATION OF FEEDING BEHAVIOUR AND WEIGHT CONTROL BY OXYNTOMODULIN

(57) Abrégé/Abstract:

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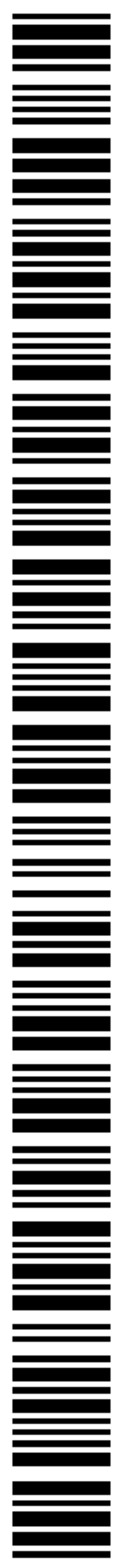
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(54) Title: MODIFICATION OF FEEDING BEHAVIOUR AND WEIGHT CONTROL BY OXYNTOMODULIN

(57) Abstract: The present invention relates to compositions and methods for use in the prevention or treatment of excess weight in a mammal. The compositions comprise oxyntomodulin which is shown to reduce food intake and/or increase energy expenditure.



**WO 2004/062685 A3**

## MODIFICATION OF FEEDING BEHAVIOUR

### INTRODUCTION

The present invention relates to compositions and methods for use in weight loss in  
5 mammalian animals.

### BACKGROUND OF THE INVENTION

One of the diseases with the highest incidence but which lacks effective treatment is  
obesity. It is a debilitating condition which reduces quality of life and substantially  
10 increases the risk of other diseases.

In the USA 25% of the adult population is now considered to be clinically obese. It  
has been estimated that \$45 billion of US healthcare costs, or 8% per annum of total  
healthcare spend, is a direct result of obesity. In Europe the problem is increasing. It  
15 has been predicted that without new approaches over 20% of the UK population will  
be clinically obese by 2005. The fact that obesity is a metabolic disease is being  
increasingly recognised by the medical profession and the health authorities. There is,  
however, a shortage of effective and safe drugs which can be used in conjunction with  
diet and exercise for the long-term management of obesity.

20

It is an object of the present invention to provide such drugs and also to provide  
means to identify and develop further such drugs.

Preproglucagon is a 160 amino acid polypeptide which is cleaved in a tissue specific  
25 manner by prohormone convertase-1 and -2 giving rise to a number of products with  
a variety of functions in both the central nervous system (CNS) and peripheral tissues.  
In the intestine and in the CNS, the major post-translational products of  
preproglucagon cleavage are glucagon-like peptide-1 (GLP-1), glucagon-like peptide-  
2 (GLP-2), glicentin and oxyntomodulin (OXM), as shown in Figure A. While GLP-  
30 1 and GLP-2 have been shown to inhibit food intake, no such role has been  
demonstrated in humans for the distinct peptide OXM. The importance of OXM as a  
biologically active peptide in humans has not been demonstrated.

## SUMMARY OF THE INVENTION

The present invention is based on our surprising observations that the OXM peptide can inhibit food intake, reduce weight and increase energy expenditure in humans,  
5 and also that OXM infusion suppresses fasting plasma ghrelin.

The present invention provides a method for the prevention or treatment of excess weight in a mammal, the method comprising administering a composition comprising OXM to a mammal. The mammal is likely to be in need of prevention or treatment of  
10 excess weight. The weight loss may be cosmetic. The composition comprising OXM will be administered in an effective concentration.

The present invention also provides the following methods of treatment of a subject: a method for decreasing calorie intake in a subject, a method for decreasing appetite  
15 in a subject, a method for decreasing food intake in a subject, a method for weight control or treatment in a subject, a method for reduction or prevention of obesity, and a method for increasing energy expenditure; in particular any one or more of the following: preventing and reducing weight gain; inducing and promoting weight loss; and reducing obesity as measured by the Body Mass Index. The methods include  
20 control of any one or more of appetite, satiety, hunger and energy expenditure, in particular any one or more of the following: reducing, suppressing and inhibiting appetite; inducing, increasing, enhancing and promoting satiety and sensations of satiety; and reducing, inhibiting and suppressing hunger and sensations of hunger; and increasing energy expenditure. The methods further include maintaining any one or  
25 more of a desired body weight, a desired Body Mass Index, a desired appearance and good health. In all the above methods OXM is administered to a subject, generally by a peripheral route of administration.

The present invention also provides a method for improving lipid profile in a subject.  
30 The method includes administering to the subject an effective amount of OXM. An improvement in lipid profile includes, but is not limited to, at least one method of reducing cholesterol levels, reducing triglyceride levels and increasing HDL

cholesterol levels. OXM can be administered peripherally, such as in a single or divided dose.

5 In another embodiment, a method is disclosed herein for alleviating a condition or disorder which can be alleviated by reducing nutrient availability and/or increasing energy expenditure. The method includes administering to a subject a therapeutically effective amount of OXM.

10 The present invention provides a pharmaceutical composition comprising OXM and a pharmaceutically suitable carrier, in a form suitable for oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation. If in unit dosage form, the dose per unit may be, for example, as described below or as calculated on the basis of the per kg doses given below.

15 The present invention also includes OXM or an agonist thereof for use in the manufacture of a medicament for administration by a route peripheral to the brain for any of the methods of treatment described above. Examples of peripheral routes include oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, 20 mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation. Preferred dose amounts of OXM for the medicaments are given below.

25 The present invention provides a method for cosmetic weight loss in a mammal, the method comprising administering a composition comprising OXM to a mammal. In this circumstance, the weight loss is purely for the purposes of cosmetic appearance.

The present invention further provides the use, in combination, of OXM and another agent that has an influence in any way on weight and/or food intake, for example, an 30 agent that has any one of more of the following effects: reduces food intake and/or reduces hunger, reduces weight, reduces or prevents obesity, increases energy expenditure or reduces nutrient availability in a mammal, especially a human. The

other agent is, for example, GLP-1 or an agonist thereof receptor, or PYY or an agonist thereof, or another substance that is or is derived from a naturally food influence substance, for example, amylin, leptin, exendin-4 or agonists thereof. If desired, more than one other agent may be used in combination with OXM, for example, GLP-1 or an agonist thereof and PYY or an agonist thereof may be used. (It will be understood that a reference to a substance "or an agonist thereof" includes mixtures of the substances and one or more agonists thereof, and also mixtures of two or more agonists.)

10 In the methods of the invention, OXM is administered in an amount effective to achieve the desired result, as is any other agent used in combination with OXM. In each case, the subject, generally a human, may be overweight and/or may be diabetic.

#### **BRIEF DESCRIPTION OF THE FIGURES**

15 Figure A is a graphical representation of preproglucagon and its component parts.

Figure 1 is a comparison of the effects of ICV and iPVN proglucagon-derived and related products on food intake in fasted rats. Figure 1A illustrates the cumulative food intake (g) up to 8 h after ICV injection of GLP-1, OXM, glucagon, or glicentin (all 3nmol) into fasted animals. \*,  $P < 0.05$  vs. saline control. Figure 1B illustrates cumulative food intake (g) up to 24 h after an acute iPVN injection of GLP-1, OXM (both 1nmol), or exendin-4 (0.03nmol) into fasted animals. \*,  $P < 0.01$  vs. saline control for all groups at 1, 2, and 4 h. \*,  $P < 0.05$  vs. saline control for exendin-4 only at 8 h.

25

Figure 2 shows two graphs of the effects of ICV and iPVN OXM on food intake in fasted rats. Figure 2A, cumulative food intake (g) up to 8 h after an acute ICV injection of OXM (0.3, 1, 3, or 10 nmol). Figure 2B, cumulative food intake (g) up to 8 h after an acute iPVN injection of OXM (0.1, 0.3, or 1.0 nmol) into fasted animals.

30 \*,  $P < 0.05$  vs. saline control.

## 5

Figure 3 shows two bar graphs of the effect of ICV OXM at the onset of the dark phase. Sated rats received an ICV injection of OXM, GLP-1 (3 nmol), or saline at the onset of the dark phase. Food intake (grams; A) and behaviors (B) at 1 h postinjection were determined. \*,  $P < 0.05$  vs. saline control.

5

Figure 4 shows two bar graphs of the inhibition of OXM and GLP-1 effects on food intake by exendin-(9-39). Figure 4A, food intake 1 h after an acute ICV injection of GLP-1 (3 nmol), GLP-1 plus exendin-(9-39) (30 nmol), OXM (3 nmol), OXM and exendin-(9-39) (30 nmol), or exendin-(9-39) alone (30 nmol). Figure 4B, food intake after an acute iPVN injection of GLP-1 (1 nmol), GLP-1 and exendin-(9-39) (10 nmol), OXM (1 nmol), OXM and exendin-(9-39) (10 nmol), or exendin-(9-39) alone (10 nmol) into fasted animals. \*\*,  $P < 0.005$  vs. saline control.

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Figure 5 is a graph of the competition of [ $^{125}$ I] GLP-1 binding in rat hypothalamic membranes by GLP-1 and OXM.

15

Figure 6 illustrates the effect of a) IP OXM (30, 100 and 300 nmol/kg in 500  $\mu$ l saline) or saline on cumulative food intake (g) in 24-hour fasted rats injected during the early dark phase (closed squares = saline, open circles = OXM 30 nmol/kg, closed triangles = OXM 100 nmol/kg, open triangles = OXM 300 nmol/kg); and b) IP OXM (30 and 100 nmol/kg in 500  $\mu$ l saline) or saline on cumulative food intake in non-fasted rats injected prior to the onset of the dark phase (closed squares = saline, open circles = OXM 30 nmol/kg, closed triangles = OXM 100 nmol/kg). \* $P < 0.05$  vs. saline.

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Figure 7 illustrates the effect of twice daily IP injections of OXM (50 nmol/kg) or saline for seven days on a) cumulative food intake (g); and b) body weight gain (g). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$  vs. saline.

Figure 8 illustrates the effect of IP OXM (50 nmol/kg), saline or a positive control (1 hour = GLP-1 (50 nmol/kg); 2 hours = CCK (15 nmol/kg)) on gastric emptying in 36-

30

hour fasted rats. Contents (dry weight) of the stomach were expressed as a percentage of the food intake during the 30-minute feeding period. \*\*P<0.01 vs. saline.

Figure 9 illustrates the effect of increasing doses of OXM (0.01 – 1.0 nmole) on 1  
5 hour food intake when administered into the arcuate nucleus of 24-hour fasted rats. \*P<0.05, \*\*P<0.01, \*\*\*P<0.05 vs. saline.

Figure 10 illustrates the effect of iARC administration of exendin 9-39 (5 nmoles) or  
saline injected 15 minutes prior to IP administration of OXM (30 nmol/kg), GLP-1  
10 (30 nmol/kg) or saline on 1 hour food intake (g). (S = saline, G = GLP-1 (30 nmol/kg), Ox = OXM (30 nmol/kg), Ex = exendin 9-39 (5 nmoles)).

Figure 11a illustrates the expression of fos-like immunoreactivity in response to A) IP  
saline or B) IP OXM (50 nmol/kg) in the arcuate nucleus of the hypothalamus (x40  
15 magnification). \*\*\*P<0.005 vs. saline; and

Figure 11b illustrates the expression of fos-like immunoreactivity in response to A) IP  
saline, B) IP OXM (50 nmol/kg) or C) IP CCK (15 nmol/kg) in the NTS and AP of  
the brainstem.

20 Figure 12 shows the protocol of the study of the effect of intravenous infusion of OXM on food intake in human subject. The scale represents time (min). Infusion of OXM (3.0 pmol/kg/min) and saline was from 0-90 minutes. The buffet meal was presented at 75 minutes.

25 Figure 13 shows the calories consumed by the human subject at the buffet meal. Each line represents the calories consumed by an individual subject with saline and OXM infusion. The bold line shows the mean calorie intake for all volunteers. The mean fall in calories with OXM infusion is  $17.6 \pm 5.7\%$ .

30 Figure 14 is a visual analogue scale showing the response of the human subjects to the question 'How hungry are you right now?' There was a significant fall in subjective

hunger during OXM infusion. Hunger scores diminished considerably following the buffet meal.

Figure 15 shows the effect of IP administration of OXM (30 nmoles/kg and 100  
5 nmoles/kg) on fasting plasma ghrelin-IR 30 and 90 minutes post-injection in rats. The solid blocks show the results with the saline control, the hatched block the results with OXM.

Figure 16 shows energy intake in kJ calories consumed by human subjects at a buffet  
10 meal. Each line represents the energy intake of an individual subject with saline and with OXM infusion. The bold line shows the mean calorie intake for all volunteers.

Figure 17 shows the energy intake at the buffet meal, and the cumulative 12 and 24  
15 hour energy intake of human subjects. The solid blocks show the results with the saline control, the hatched block the results with OXM.

Figure 18 shows the relative hunger scores of the human subjects during a fasting  
period and after a meal, with infusion of OXM or a saline control for the period  
shown.

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Figure 19 shows the OXM-like immunoreactivity (OLI) in pmol/L determined by an  
RIA during a fasting period and after a meal, with infusion of OXM or a saline  
control for the period shown.

25 Figure 20 shows gel permeation analysis of plasma samples during OXM infusion.  
The single immunoreactive peak elutes at the same position as synthetic OXM.

Figure 21 shows the change in plasma ghrelin levels during a fasting period and after  
a meal, with infusion of OXM or a saline control for the period shown.

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**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based on the surprising observation that, found that contrary to expectations, the OXM peptide can inhibit food intake and reduce weight.

- 5 In this text, the term “oxyntomodulin” is the same as "OXM" and relates to any composition which includes an OXM peptide sequence or an analogue thereof as follows:

OXM sequences are well known and documented in the art. The present invention  
10 relates to all of the sequences recited herein including, in particular, the OXM human sequence SEQ ID NO: 1 (which is the same as the rat, hamster and bovine OXM sequence), as follows:

|    |     |     |     |     |     |     |     |              |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|--------------|-----|-----|
|    | His | Ser | Gln | Gly | Thr | Phe | Thr | Ser          | Asp | Tyr |
| 15 | Ser | Lys | Tyr | Leu | Asp | Ser | Arg | Arg          | Ala | Gln |
|    | Asp | Phe | Val | Gln | Trp | Leu | Met | Asn          | Thr | Lys |
|    | Arg | Asn | Lys | Asn | Asn | Ile | Ala | SEQ ID NO: 1 |     |     |

the OXM angler fish sequence SEQ ID NO: 2 as follows:

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|  |     |     |     |     |     |     |              |     |     |     |
|--|-----|-----|-----|-----|-----|-----|--------------|-----|-----|-----|
|  | His | Ser | Glu | Gly | Thr | Phe | Ser          | Asn | Asp | Tyr |
|  | Ser | Lys | Tyr | Leu | Glu | Asp | Arg          | Lys | Ala | Gln |
|  | Glu | Phe | Val | Arg | Trp | Leu | Met          | Asn | Asn | Lys |
|  | Arg | Ser | Gly | Val | Ala | Glu | SEQ ID NO: 2 |     |     |     |

25

and the eel OXM sequence SEQ ID NO: 3 as follows:

|    |     |     |     |     |     |     |              |     |     |     |
|----|-----|-----|-----|-----|-----|-----|--------------|-----|-----|-----|
|    | His | Ser | Gln | Gly | Thr | Phe | Thr          | Asn | Asp | Tyr |
| 30 | Ser | Lys | Tyr | Leu | Glu | Thr | Arg          | Arg | Ala | Gln |
|    | Asp | Phe | Val | Gln | Trp | Leu | Met          | Asn | Ser | Lys |
|    | Arg | Ser | Gly | Gly | Pro | Thr | SEQ ID NO: 3 |     |     |     |

The term OXM used in this text also covers any analogue of the above OXM sequence, wherein the histidine residue at position 1 is maintained or replaced by an aromatic moiety carrying a positive charge or a derivative thereof, preferably wherein the moiety is an amino acid, more preferably wherein it is a histidine derivative, while  
5 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 of the other amino acids in the above OXM sequence can be independently replaced by any other independently chosen amino acid, with the exception of histidine in position 1.

Any one or more (to 22) other alpha-amino acid residue in the sequence can be  
10 independently replaced by any other one alpha-amino acid residue. Preferably, any amino acid residue other than histidine is replaced with a conservative replacement as well known in the art i.e. replacing an amino acid with one of a similar chemical type such as replacing one hydrophobic amino acid with another.

15 As discussed above, 1 to 22 of the amino acids can be replaced. In addition to the replacement option above, this may be by a non-essential or modified or isomeric form of an amino acid. For example, 1 to 22 amino acids can be replaced by an isomeric form (for example a D-amino acid), or a modified amino acid, for example a nor-amino acid (such as norleucine or norvaline) or a non-essential amino acid (such  
20 as taurine). Furthermore, 1 to 22 amino acids may be replaced by a corresponding or different amino acid linked via its side chain (for example gamma-linked glutamic acid). For each of the replacements discussed above, the histidine residue at position 1 is unaltered or defined above.

25 In addition, 1, 2, 3, 4 or 5 of the amino acid residues can be removed from the OXM sequence with the exception of histidine at the 1 position (or as defined above). The deleted residues may be any 2, 3, 4 or 5 contiguous residues or entirely separate residues.

30 The C-terminus of the OXM sequence may be modified to add further amino acid residues or other moieties. The OXM above may be provided as the corresponding salt thereof. Examples of pharmaceutically acceptable salts of OXM and its

analogues include those derived from organic acids such as methanesulphonic acid, benzenesulphonic acid and p-toluenesulphonic acid, mineral acids such as hydrochloric and sulphuric acid and the like, giving methanesulphonate, benzenesulphonate, p-toluenesulphonate, hydrochloride and sulphate, and the like, respectively or those derived from bases such as organic and inorganic bases. Examples of suitable inorganic bases for the formation of salts of compounds for this invention include the hydroxides, carbonates, and bicarbonates of ammonia, lithium, sodium, calcium, potassium, aluminium, iron, magnesium, zinc and the like. Salts can also be formed with suitable organic bases. Such bases suitable for the formation of pharmaceutically acceptable base addition salts with compounds of the present invention include organic bases which are nontoxic and strong enough to form salts. Such organic bases are already well known in the art and may include amino acids such as arginine and lysine, mono-, di-, or trihydroxyalkylamines such as mono-, di-, and triethanolamine, choline, mono-, di-, and trialkylamines, such as methylamine, dimethylamine, and trimethylamine, guanidine; N-methylglucosamine; N-methylpiperazine; morpholine; ethylenediamine; N-benzylphenethylamine; tris(hydroxymethyl) aminomethane; and the like.

Salts may be prepared in a conventional manner using methods well known in the art. Acid addition salts of said basic compounds may be prepared by dissolving the free base compounds in aqueous or aqueous alcohol solution or other suitable solvents containing the required acid. Where OXM contains an acidic function a base salt of said compound may be prepared by reacting said compound with a suitable base. The acid or base salt may separate directly or can be obtained by concentrating the solution eg. by evaporation. OXM may also exist in solvated or hydrated forms.

The OXM of the present invention may be conjugated to one or more groups such as a lipid, sugar, protein or polypeptide. The OXM can be conjugated by being attached to the group (for example via a covalent or ionic bond) or can be associated therewith. The conjugated link is preferably not through the C or N terminus amino acid, when the OXM is attached to the group. The OXM can be conjugated to a polymer such as polyethylene glycol, polyvinylpyrrolidone, polyvinylalcohol, polyoxyethylene-

polyoxypropylene copolymers, polysaccharides such as cellulose, cellulose derivatives, chitosan, acacia gum, karaya gum, guar gum, xanthan gum, tragacanth, alginic acid, carrageenan, agarose, and furcellarans, dextran, starch, starch derivatives, hyaluronic acid, polyesters, polyamides, polyanhydrides, and polyortho esters.

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The OXM can be chemically modified. In particular, the amino acid side chains, the N terminus and/or the C acid terminus of OXM can be modified. For example, the OXM can undergo one or more of alkylation, disulphide formation, metal complexation, acylation, esterification, amidation, nitration, treatment with acid, treatment with base, oxidation or reduction. Methods for carrying out these processes are well known in the art. In particular the OXM is provided as a lower alkyl ester, a lower alkyl amide, a lower dialkyl amide, an acid addition salt, a carboxylate salt or an alkali addition salt thereof. In particular, the amino or carboxylic termini of the OXM may be derivatised by for example, esterification, amidation, acylation, oxidation or reduction. In particular, the carboxylic terminus of the OXM can be derivatised to form an amide moiety.

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The OXM can be treated with metals, in particular with divalent metals. For the purposes of this invention the OXM can therefore be provided in the presence of one or more of the following metals, zinc, calcium, magnesium, copper, manganese, cobalt, molybdenum or iron.

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The OXM can be provided in the form of a pharmaceutical composition in combination with a pharmaceutically acceptable carrier or diluent. Suitable carriers and/or diluents are well known in the art and include pharmaceutical grade starch, mannitol, lactose, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, (or other sugar), magnesium carbonate, gelatin, oil, alcohol, detergents, emulsifiers or water (preferably sterile). The composition may be a mixed preparation of a composition or may be a combined preparation for simultaneous, separate or sequential use (including administration). The OXM can be provided as a crystalline solid, a powder, an aqueous solution, a suspension or in oil.

The compositions according to the invention for use in the aforementioned indications may be administered by any convenient method, for example by oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including  
5 administration by inhalation, and the compositions adapted accordingly.

For oral administration, the composition can be formulated as liquids or solids, for example solutions, syrups, suspensions or emulsions, tablets, capsules and lozenges.

10 A liquid formulation will generally consist of a suspension or solution of the compound or physiologically acceptable salt in a suitable aqueous or non-aqueous liquid carrier(s) for example water, ethanol, glycerine, polyethylene glycol or an oil. The formulation may also contain a suspending agent, preservative, flavouring or colouring agent.

15 A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and microcrystalline cellulose.

20 A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, powders, granules or pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable  
25 pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule.

Compositions for oral administration may be designed to protect the active ingredient against degradation as it passes through the alimentary tract, for example by an outer  
30 coating of the formulation on a tablet or capsule.

Typical parenteral compositions, including compositions for subcutaneous administration, comprise a solution or suspension of the compound or physiologically acceptable salt in a sterile aqueous or non-aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration.

Compositions for nasal or oral administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal once the contents of the container have been exhausted. Where the dosage form comprises an aerosol dispenser, it will contain a pharmaceutically acceptable propellant. The aerosol dosage forms can also take the form of a pump-atomiser.

Compositions suitable for buccal or sublingual administration include tablets, lozenges and pastilles, wherein the active ingredient is formulated with a carrier such as sugar and acacia, tragacanth, or gelatin and glycerin.

Compositions for rectal or vaginal administration are conveniently in the form of suppositories (containing a conventional suppository base such as cocoa butter), pessaries, vaginal tabs, foams or enemas.

Compositions suitable for transdermal administration include ointments, gels, patches and injections including powder injections.

30

Conveniently the composition is in unit dose form such as a tablet, capsule or ampoule.

OXM may be administered peripherally at a dose of, for example, 0.1 nmoles or more per kg body weight of the subject, for example, 0.2 nmoles or more, for example, 0.5 nmoles or more, for example, 1 nmole or more, for example, 1.5 nmoles or more, for example, 2 nmole or more, for example, 2.5 nmoles or more, for example, 3 nmoles or more, for example, 4 nmoles or more, for example, 5 nmoles or more, for example, 6 nmoles or more, for example, 7 nmoles or more, for example, 8 nmoles or more, for example, 9 nmoles or more, for example, 10 nmoles , for example, 11 nmoles or more, for example, up to 12 nmoles per kg body weight. The amount used may be up to 11 nmoles per kg body weight, for example, up to 10 nmoles, for example, up to 9 nmoles, for example, up to 8 nmoles, for example, up to 7 nmoles, for example, up to 6 nmoles, for example, up to 5 nmoles, for example, up to 4 nmoles, for example, up to 3 nmoles, for example, up to 2 nmoles, for example, up to 1 nmoles, for example, up to 0.5 nmoles, for example, up to 0.4 nmoles, for example, up to 0.2 nmoles per kg body weight. The dose is generally in the range of from 0.1 to 12 nmoles per kg body weight, for example, within any combination of upper and lower ranges given above. A dose may be calculated on an individual basis or on the basis of a typical subject, often a 70 or 75 kg subject. The dose may be administered before each meal.

For subcutaneous administration, a dose of OXM within the range of from 100nmol to 500 nmol i.e. about 0.5mg to about 2mg, which dose is calculated on the basis of a 75 kg subject, may be administered, generally before meals.

A pharmaceutical preparation in unit dosage form for peripheral administration preferably comprises an amount of OXM calculated on the basis of the per kg doses given above. Typically, the dose may be calculated on the basis of a 70 or 75kg subject. A composition for subcutaneous administration, for example, may comprise a unit dose of OXM within the range of from 100nmol to 500 nmol i.e. about 0.5mg to about 2mg, calculated on the basis of a 75 kg subject.

The OXM can be used as a prophylaxis to prevent excess weight gain or can be used as a therapeutic to lose excess weight.

5 The excess weight is typically obesity, although the mammal will not be certified as clinically obese in order to be suffering from excess weight. The OXM may be in liquid, solid or semi-solid form.

10 In today's society, the prevention or treatment of excess weight in a mammal is a real need. Preferably the mammal is a human, although it may also include other mammalian animals, such as horses, canine animals (in particular domestic canine animals), feline animals (in particular domestic feline animals) as well as mammals which are produced for meat, such as porcine, bovine and ovine animals. The present invention can be used to prevent excess weight in such animals in order to maximise lean meat production.

15

Throughout this text, the term "prevention" means any effect which mitigates any excess weight, to any extent. Throughout this text, the term "treatment" means amelioration of excess weight, to any extent.

20 Suitable doses of OXM include those that raise the concentration of OXM significantly above the basal concentration of OXM, such as, but not limited to, a dose that that mimic postprandial serum concentrations of OXM. Thus, in one embodiment, OXM is administered to a reduction in calorie intake, food intake, or appetite equivalent to the reduction in calorie intake, food intake, or appetite, or to  
25 increase the energy expenditure, caused by the postprandial level of OXM.

For all methods disclosed herein, the dose of OXM can be based on the physiological levels observed post-prandially. A single dose may be administered per day, or divided doses can be used (see above).

30

It is preferable to administer OXM via a peripheral route of administration, that is to say, via a route other than directly to the brain. Examples of such routes include oral,

rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation.

- 5 The present invention provides a pharmaceutical composition comprising OXM and a pharmaceutically suitable carrier, in a form suitable for oral, rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation. If in unit dosage form, the dose may per unit may be calculated on the basis of the per  
10 kg doses given above.

- The present invention also includes OXM or an agonist thereof for use in the manufacture of a medicament for administration by a peripheral route for any of the methods of treatment described above. Examples of peripheral routes include oral,  
15 rectal, parenteral eg intravenous, intramuscular, or intraperitoneal, mucosal e.g. buccal, sublingual, nasal, subcutaneous or transdermal administration, including administration by inhalation. Preferred dose amounts of OXM for the medicaments are given above.

- 20 The present invention provides a method for cosmetic weight loss in a mammal, the method comprising administering a composition comprising OXM to a mammal. In this circumstance, the weight loss is purely for the purposes of cosmetic appearance.

All preferred features given above apply to this aspect of the invention.

25

- Without being bound to this theory, it is understood that the present invention provides the prevention or treatment of excess weight by the administration of OXM which acts as an inhibitor to food intake to the mammalian body and/or increases energy expenditure. Such reduced food intake and/or increased energy expenditure  
30 results in the prevention or treatment of excess weight in a mammal. In this text the term "food" includes a substance which is ingested and which has calorific value. Furthermore, we have found that OXM infusion suppresses fasting plasma ghrelin.

This is an important finding because ghrelin is a powerful stimulant of appetite in man and preprandial rises in plasma ghrelin have been suggested to be a trigger for meal initiation. Without being bound by the hypothesis, we consider that inhibition of the normal preprandial rise in ghrelin by OXM is likely to be one mechanism by which  
5 OXM infusion reduces appetite.

The present invention further provides the use, in combination, of OXM and another agent that has an influence in any way on weight and/or food intake, for example, an agent that has any one of more of the following effects: reduces food intake and/or  
10 reduces hunger, reduces weight, reduces or prevents obesity, increases energy expenditure or reduces nutrient availability in a mammal, especially a human. The other agent is, for example, GLP-1 or an agonist thereof receptor, or PYY or an agonist thereof, or another substance that is or is derived from a naturally food influence substance, for example, amylin, leptin, exendin-4 or agonists thereof. If  
15 desired, more than one other agent may be used in combination with OXM, for example, GLP-1 or an agonist thereof and PYY or an agonist thereof may be used. (It will be understood that a reference to a substance "or an agonist thereof" includes mixtures of the substances and one or more agonists thereof, and also mixtures of two or more agonists.)

20

In one embodiment OXM may be used with GLP-1 or an agonist thereof. OXM appears to have an arcuate site of action, whereas GLP-1 acts via the brain stem. The use of the two agents in combination may give a synergistic effect.

25 GLP-1, like OXM, is a post-translational product of preproglucagon, see Figure A. The initial post-translational product is GLP-1 (1-37). Human GLP-1 (1-37) has the following amino acid sequence, SEQ ID NO: 4:

His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu  
30 Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
SEQ ID NO: 4

Further modifications give GLP-1 (1-36) SEQ.ID.NO: 5, and the amide thereof GLP-1 (1-36) NH<sub>2</sub>; GLP-1 (7-37) SEQ.ID.NO:6; and GLP-1 (7-36) SEQ.ID.NO:7 and the amine thereof, GLP-1 (7-36) NH<sub>2</sub>, which is the most biologically active of the GLP-1 peptides. The term "GLP-1" is used herein to denote any of the GLP-1 peptides  
5 defined above, especially GLP-1 (7-36) NH<sub>2</sub>, also known as GLP-1 (7-36) amide. The terms encompasses GLP-1 peptides of any animal origin, especially the human peptides.

A GLP-1 agonist is a peptide, small molecule, or chemical compound that  
10 preferentially binds to the GLP-1 receptor and stimulates the same biological activity as does GLP-1. In one embodiment, an agonist for the GLP-1 receptor binds to the receptor with an equal or greater affinity than GLP-1. In another embodiment, an agonist selectively binds the GLP-1 receptor, as compared to binding to another  
15 receptor. Exendin-4, which is a 39-amino acid peptide isolated from the salivary glands of the Gila monster (*Heloderma suspectum*) (Eng J et al J Biol Chem 267:7402-7405, 1992) is an example of an agonist at the GLP-1 receptor. Molecules derived from exendin-4 and that also have GLP-1 agonist activity are further  
examples of GLP-1 agonists. GLP-1 agonists include GLP-1 related peptides and peptides that result from natural or synthetic enzymatic or chemical processing of  
20 preproglucagon or of a GLP-1 peptide or a related peptide.

Any compound that is described as being a GLP-1 agonist may be used in the present invention, as may any compound that is tested for GLP-1 agonist activity, for example, as described above, and found to function as a GLP-1 agonist. A  
25 recombinant GLP-1 receptor suitable for use in screening is disclosed in WO93/19175. Many GLP-1 agonists are known and are described in the art. Examples of published patent specifications that disclose GLP-1 agonists are the following: WO2002/67918, WO2002/66479, WO2002/03978, WO2001/89554, WO2001/14386, WO2001/66135, WO2001/35988, WO2001/14368, WO2001/04156,  
30 WO2000/78333, WO2000/59887, WO2000/42026, EP 0955314, and WO99/43707. Examples of GLP-1 agonists are Arg34, Lys26(N-epsilon-(gamma-Glu(N-alpha-hexadecanoyl)))-GLP-1 (7-37), IP7-GLP-1 (7-37)OH.

It may be advantageous to use PYY or an agonist thereof with OXM. PYY has a sustained duration of action, for example, when administered peripherally, it continues to act after it has been cleared from the circulating blood, for example, for up to 24 hours after administration. Accordingly, PYY is effective when two or even one dose per day is administered. Without being limited by the following, OXM appears to have an immediate effect, which may not be sustained for a prolonged period. OXM may be administered several times per day, for example, before a meal. The use of long acting PYY with short acting OXM enables "fine tuning" of administration regimes to the needs of the user.

PYY is a 36-residue peptide amide isolated originally from porcine intestine (Tatemoto et al. Proc. Natl. Acad. Sci. 79:2514, 1982). The term as used herein includes PYY obtained or derived from any species. Thus, PYY includes the human full length polypeptide, which has the following sequence, SEQ ID NO: 8:

Tyr Pro Ile Lys Pro Glu Ala Pro Gly Glu Asp Ala Ser Pro Glu Glu Leu Asn Arg Tyr  
Tyr Ala Ser Leu Arg His Tyr Leu Asn Leu Val Thr Arg Gln Arg Tyr SEQ ID NO: 8

and species variations of PYY, including e.g. murine, hamster, chicken, bovine, rat, and dog. In one embodiment, PYY agonists do not include NPY. The term PYY as used herein also includes PYY<sub>3-36</sub>. It may be advantageous to use PYY<sub>3-36</sub>. A PYY agonist is any compound which binds to a receptor that specifically binds PYY, and elicits an effect of PYY. In one embodiment, a PYY agonist is a compound that affects food intake, caloric intake, or appetite, and/or which binds specifically in a Y receptor assay or competes for binding with PYY, such as in a competitive binding assay with labeled PYY. PYY agonists include, but are not limited to, compounds that bind to the Y2 receptor.

PYY agonists and compounds that may be used as PYY agonists are disclosed in the art. For example, contemplated as useful PYY agonists are Y2 specific NPY peptide agonists as described in U.S. Patent No. 5,026,685; U.S. Patent No. 5,574,010; U.S.

Patent No. 5,604,203; U.S. Patent No. 5,696,093; U.S. Patent No. 6,046,167. There may also be used variants of PYY and of neuropeptide Y that are analogous to the variants and modifications of OXM described above.

- 5 If desired, OXM may be used in with both GLP-1 or an agonist thereof and PYY or an agonist thereof.

The use of a combination of any of OXM and GLP-1 or an agonist thereof and PYY or an agonist thereof may serve to increase the effectiveness of any of the agents compared with its use alone, for example, as described above. Alternatively or in addition, use of the two or three agents in combination may reduce any tendency for “escape” when using an agent alone. The term “escape” is used to denote a reduction in effect of an agent with time. For example, if any one of the agents above has been used alone, its effect may reduce with time. Use of one or both of the other agents in addition may reduce or prevent the tendency for that reduction in effectiveness. For example, PYY has a sustained effect and may be used for prolonged periods. If the effect of PYY should appear to reduce, or to reduce or prevent any such reduction in effect, OXM may be administered in addition to the PYY. GLP-1 may also be used for the same purpose, with OXM or with OXM and PYY.

20

If desired, one or more other agents, such as, but not limited to, an additional appetite suppressant, may also be administered. Specific, non-limiting example of an additional appetite suppressant include amfepramone (diethylpropion), phentermine, mazindol and phenylpropanolamine, fenfluramine, dexfenfluramine, and fluoxetine.

25

When used in combination with another agent, OXM may be administered simultaneously or substantially simultaneously as the other agent, or sequentially, in either order. OXM and the other agent may be administered in a single pharmaceutical composition or in separate compositions, and they may be administered by the same route or by different routes. It is generally more convenient to administer all the active agents in a single composition. However, in some cases it may be necessary or appropriate to administer the active agents by different routes.

30

For example, peptides are generally not stable on oral administration unless modified or formulated in a special way, so must generally be administered via a non-oral route. Some agonists, for example, GLP-1 agonists, are chemical compounds that are stable when administered orally. It may be appropriate to administer OXM non-orally  
5 and the other component by a non-oral route.

According to a preferred aspect of the invention, a therapeutically effective amount of OXM or an agonist thereof is administered with a therapeutically effective amount of GLP-1 or an agonist thereof and/or PYY or an agonist thereof. The term “GLP-  
10 1/PYY” is used herein to denote GLP-1 or an agonist thereof and/or PYY or an agonist thereof.

The OXM or agonist thereof and the GLP-1/PYY may be administered simultaneously or substantially simultaneously, or sequentially, in any order. The  
15 OXM or agonist thereof and the GLP-1/PYY may be administered in a single pharmaceutical composition or in separate compositions, and they may be administered by the same route or my different routes.

If the OXM and the GLP-1/PYY are to be administered in a single pharmaceutical  
20 composition, that composition may be any of those described above for OXM or an agonist thereof. The composition may enable simultaneous or substantially simultaneous administration of the OXM or agonist thereof and the GLP-1/PYY. If desired, the OXM or agonist thereof and the GLP-1/PYY may be compartmentalized in the composition, for example, in different layers of a tablet, or in different granules  
25 in a capsule. If desired, such compartmentalization may be designed to give different release properties to the components to enable delivery of the OXM or agonist component and the GLP-1/PYY at different times, for example, sequentially.

Alternatively, the OXM or agonist thereof and the GLP-1/PYY may be formulated in  
30 separate pharmaceutical compositions, for example, any of the pharmaceutical

compositions described above for OXM and agonists thereof. Such separate compositions may be administered simultaneously or substantially simultaneously, or they may be administered sequentially, in any order. For example, PYY may be administered two times or even once per day, with OXM being administered up to  
5 several times per day, for example, before meals.

If administered separately, whether sequentially or simultaneously (or substantially simultaneously), the OXM or agonist thereof and the GLP-1/PYY may be administered by the same route or by different routes, for example, as described  
10 above.

When used in combination therapy as described above, OXM may be used in a dose as disclosed above in relation to peripheral administration when used alone, that is to say, OXM may be administered peripherally at a dose of, for example, 0.1 nmoles or  
15 more per kg body weight of the subject, for example, 0.2 nmoles or more, for example, 0.5 nmoles or more, for example, 1 nmole or more, for example, 1.5 nmoles or more, for example, 2 nmole or more, for example, 2.5 nmoles or more, for example, 3 nmoles or more, for example, 4 nmoles or more, for example, 5 nmoles or more, for example, 6 nmoles or more, for example, 7 nmoles or more, for example, 8  
20 nmoles or more, for example, 9 nmoles or more, for example, 10 nmoles, for example, 11 nmoles or more, for example, up to 12 nmoles per kg body weight. The amount used may be up to 11 nmoles per kg body weight, for example, up to 10 nmoles, for example, up to 9 nmoles, for example, up to 8 nmoles, for example, up to 7 nmoles, for example, up to 6 nmoles, for example, up to 5 nmoles, for example, up  
25 to 4 nmoles, for example, up to 3 nmoles, for example, up to 2 nmoles, for example, up to 1 nmoles, for example, up to 0.5 nmoles, for example, up to 0.4 nmoles, for example, up to 0.2 nmoles per kg body weight. The dose is generally in the range of from 0.1 to 12 nmoles per kg body weight, for example, within any combination of upper and lower ranges given above.

30

GLP-1 or an agonist thereof may be administered peripherally at a dose of, for example, 0.1 nmoles or more per kg body weight of the subject, for example, 0.2 -

nmols or more, for example, 0.4 nmols or more, for example, 0.6 nmols or more, for example, 0.8 nmols or more, for example, 1.0 nmole or more, for example, 1.2 nmols or more, for example, 1.4 nmols or more, for example, 1.6 nmols or more, for example, 1.8 nmols or more, for example, 2.0 nmols or more, for example, 2.2 nmols or more, for example, 2.4 nmols or more, for example, 2.6 nmols or more, for example, 2.8 nmols , for example, 3.0 nmols or more, for example, up to 3.2 nmols per kg body weight. The amount used may be up to 3.0 nmols per kg body weight, for example, up to 2.8 nmols, for example, up to 2.6 nmols, for example, up to 2.4 nmols, for example, up to 2.2 nmols, for example, up to 2.0 nmols, for example, up to 1.8 nmols, for example, up to 1.4 nmols, for example, up to 1.2 nmols, for example, up to 1.0 nmols, for example, up to 0.8 nmols, for example, up to 0.6 nmols, for example, up to 0.4 nmols, for example, up to 0.2 nmols per kg body weight. The dose is generally in the range of from 0.1 to 3.2 nmols per kg body weight, for example, within any combination of upper and lower ranges given above.

PYY or an agonist thereof may be used at a dose within the ranges disclosed above for GLP-1. The doses of the various agent may be independent of each other or, for example, equimolar doses may be used, for example, equimolar doses of GLP-1 or an agonist thereof and PYY or an agonist thereof. A dose may be calculated on an individual basis or on the basis of a typical subject, often a 70 or 75 kg subject.

A further embodiment of the present invention is a pharmaceutical composition comprising oxyntomodulin and one or more other agents having an influence in any way on weight and/or food intake, for example, an agent that has any one of more of the following effects: reduces food intake and/or reduces hunger, reduces weight, reduces or prevents obesity, increases energy expenditure or reduces nutrient availability in a mammal, especially a human, in admixture or conjunction with a pharmaceutically suitable carrier. The agents are as defined above and are, for example, GLP-1 or an agonist and/or PYY agonist thereof. The compositions may be, for example, as described above for OXM pharmaceutical compositions. Doses of the OXM and other agents are, for example, as described above.

A pharmaceutical preparation in unit dosage form for peripheral administration preferably comprises an amount of OXM calculated on the basis of the per kg doses given above. Typically, the dose may be calculated on the basis of a 75kg subject.

- 5 A composition for subcutaneous administration, for example, may comprise a unit dose of OXM within the range of from 100nmol to 500 nmol i.e. about 0.5mg to about 2mg, calculated on the basis of a 75 kg subject.

The present invention also provides the use of OXM in the manufacture of a  
10 medicament for the treatment of a subject according to any of the methods disclosed above.

When OXM and another agent that reduces food intake, for example, PYY or an agonist thereof and/or GLP-1 or an agonist thereof are used in the manufacture of a  
15 medicament for use in a treatment as described herein, the medicament may be a single pharmaceutical composition comprising all the components, as described above, or may be a two or more component medicament, one component being a pharmaceutical composition comprising OXM, the other component(s) each being a pharmaceutical composition comprising the other agent(s) that reduce food intake, see  
20 above.

The medicament, whether a one component medicament or a two or more component medicament as described above, will generally be packaged with instructions relating to its use. Such instructions will refer to the timing, dose and route of administration  
25 of the component(s).

The preferred features above relating to methods and compositions relating to OXM when used in combination with other agent also applies to its use in the manufacture of a medicament as described above.

30

In all embodiments of the invention, the particular dosage regime for which will ultimately be determined by the attending physician and will take into consideration

such factors as the OXM being used, animal type, age, weight, severity of symptoms and/or severity of treatment to be applied, method of administration of the medicament, adverse reaction and/or contra indications. Specific defined dosage ranges can be determined by standard designed clinical trials with patient progress  
5 and recovery being fully monitored.

Such trials may use an escalating dose design using a low percentage of the maximum tolerated dose in animals as the starting dose in man. Examples of suitable doses are given above.

10 Preferred features of each aspect of the invention are as for each of the other aspects mutatis mutandis.

The present invention is now described by way of example only in the following non-  
15 limiting Examples.

## EXAMPLES

### Example 1

OXM causes a potent decrease in fasting-induced refeeding when injected both ICV  
20 and iPVN

#### Peptides and chemicals

GLP-1, glicentin, glucagon, and SP-1 were purchased from Peninsula Laboratories, Inc. (St. Helens, UK). OXM was purchased from IAF BioChem Pharma (Laval,  
25 Canada). Exendin-4 and exendin-(9-39) were synthesised at Medical Research Council, Hemostasis Unit, Clinical Sciences Center, Hammersmith Hospital, London, UK using F-moc chemistry on an 396 MPS peptide synthesiser (Advanced ChemTech, Inc.) and purified by reverse phase HPLC on a C<sub>8</sub> column (Phenomex, Macclesfield, UK). The correct molecular weight was confirmed by mass  
30 spectrometry. All chemicals were purchases from Merck & Co. (Lutterworth, Leicester, UK) unless otherwise stated.

## Animals

Adult male Wistar rats (ICSM, Hammersmith Hospital) were maintained in individual cages under controlled conditions of temperature (21-23°C) and light (12h of light, 12h of darkness) with ad libitum access to food (RM1 diet, Special Diet Services UK Ltd., Witham, UK) and tap water. Animals were handled daily after recovery from surgery until completion of the studies. All animal procedures undertaken were approved by the British Home Office Animals (Scientific Procedures Act 1986 (Project License PIL 90/1077)).

## 10 ICV and iPVN cannulation and infusions of test compounds

Animals had permanent stainless steel guide cannulas (Plastics One, Roanoke, VA) stereotactically implanted ICV (intracerebraventricularly) or iPVN (into the hypothalamic paraventricular nucleus). All studies were carried out in the early light phase, between 0900-1100h, after a 24-h fast, and food intake was measured 1, 2, 4, 8, and 24h postinjection.

## Feeding study protocols

Comparison of the effect of proglucagon-derived products and related peptides on food intake.

20

In study 1a, rats were injected ICV with 10µl saline, GLP-1 (13 nmol), OXM (3 nmol), glucagon (3 nmol), or glicentin (3 nmol; n= 8/group).

In all studies, the human OXM with the following sequence, SEQ ID NO: 1, was used:

25

|     |     |     |     |     |     |     |              |     |     |
|-----|-----|-----|-----|-----|-----|-----|--------------|-----|-----|
| His | Ser | Gln | Gly | Thr | Phe | Thr | Ser          | Asp | Tyr |
| Ser | Lys | Tyr | Leu | Asp | Ser | Arg | Arg          | Ala | Gln |
| Asp | Phe | Val | Gln | Trp | Leu | Met | Asn          | Thr | Lys |
| Arg | Asn | Lys | Asn | Asn | Ile | Ala | SEQ ID NO: 1 |     |     |

30

Human GLP-1 with the following sequence, SEQ ID NO: 7, was used:

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Ala | Glu | Gly | Thr | Phe | Thr | Ser | Asp | Val |
| Ser | Ser | Tyr | Leu | Glu | Gly | Gln | Ala | Ala | Lys |
| Glu | Phe | Ile | Ala | Trp | Leu | Val | Lys | Gly | Arg |

5 SEQ ID NO: 7.

In study 1b, rats were injected iPVN with 1 $\mu$  saline, GLP-1 (1.0 nmol), OXM (1.0 nmol), glicentin (1.0 nmol), glucagon (1.0 nmol), or SP-1 (3.0 nmol; n = 12-15/group). Exendin-4, when injected ICV, inhibits food intake more potently than  
10 GLP-1. Therefore, exendin-4 was injected iPVN at a dose of 0.03 nmol.

Investigation of the effect of increasing doses of OXM on food intake

In study 2a, rats were injected ICV with saline, GLP-1 (3nmol), or OXM (0.3, 1, 3 or 10 nmol; n = 8/group). In study 2b, rats were injected iPVN with saline, GLP-1 (1.0  
15 nmol), or OXM (0.1, 0.3, or 1.0 nmol; n = 12-15/group). To assess whether OXM acts via the GLP-1 receptor, a study using the GLP-1 receptor antagonist exendin-(9-39) was performed.

Night time feeding and behavioural analysis.

20 Study 3. It is possible that OXM inhibits food intake via nonspecific taste aversion, and that it is not a true satiety factor. Therefore, ICV cannulated rats were administered GLP-1 (3nmol), OXM (3 nmol), or saline (n = 6/group) at the onset of the dark phase. Food intake was measured 1 h postinjection (study 3a), and behaviour was assessed (study 3b). Rats were observed for 1 h postinjection using a behavioural  
25 score sheet.

In study 4a, rats were injected with ICV with saline, GLP-1 (3nmol), GLP-1 (3nmol) plus exendin-(9-39) (30 nmol), OXM (3 nmol), OXM (3 nmol) plus exendin-(9-39) (30 nmol), or exendin-(9-39) alone (30 nmol). In study 4b, rats were iPVN injected  
30 with saline, GLP-1 (1 nmol), GLP-1 (1nmol) plus exendin-(9-39) (10 nmol), OXM (1 nmol), OXM (1 nmol) plus exendin-(9-39) (10 nmol), or exendin-(9-39) alone (10 nmol; n = 10-12/group).

Receptor binding assays. Study 5.

Receptor binding assays were performed in a final volume of 0.5 ml rat hypothalamic membranes (200µg protein), 500 Bq (100pM) [<sup>125</sup>I]GLP-1, and unlabeled competing peptides (GLP-1 and OXM) as specified. Membranes were incubated at room temperature for 90 min. Bound and free radioactivity were separated by centrifugation (2 min, 4°C). Pelleted membranes were washed with assay buffer (0.5 ml, ice-cold), and the membranes were centrifuged as described above. The supernatant was removed, and the radioactivity in the pellet was counted using a γ-counter. Specific (saturable) binding was calculated as the difference between the amount of [<sup>125</sup>I]GLP-1 bound in the absence (total binding) and presence of 1µM GLP-1 or OXM (nonsaturable binding). All curves were constructed with points in triplicate. IC<sub>50</sub> values were calculated using the Prism 3 program (GraphPad Software, Inc., San Diego, CA).

#### Statistics

For food intake analyses, data are presented as the mean ± SEM. Statistical differences between experimental groups were determined by ANOVA, followed by a post-hoc least significant difference test (Systat 8.0, Evanston, IL). For behavioural analyses, data are expressed as the median number of occurrences of each behaviour and the range. Comparisons between groups were made using the Mann-Whitney U test (Systat 8.0). In all cases, P<0.05 was considered statistically significant.

#### Results

Comparison of the effects of proglucagon-derived products and related peptides on food intake

#### ICV administration.

In study 1a, OXM and GLP-1 (3 nmol) significantly reduced refeeding. This inhibition of food intake lasted until 4h postinjection (Fig. 1A). Glucagon and glicentin (3nmol) failed to affect food intake at any time point (Fig. 1A).

iPVN administration.

In study 1b, OXM, GLP-1 (3 nmol) and exendin-4 (0.03nmol) also inhibited refeeding when injected iPVN. This inhibition lasted at least 8h postinjection, longer than when injected ICV (Fig. 1B). Glicentin, glucagon (1 nmol), and SP-1 (3 nmol) failed to affect food intake at any time point when injected iPVN.

Effects of increasing doses of OXM on food intake

ICV administration.

In study 2a, when injected ICV, OXM reduced refeeding in a dose-dependent manner, reaching a maximal effect at a dose of 3 nmol 1, 2, and 4h postinjection (Fig. 2A).

iPVN administration.

In study 2b, food intake was significantly reduced by iPVN-injected GLP-1 and OXM (both 1 nmol) until 8h postinjection (Fig. 2B).

Effect of OXM in ICV-cannulated sated rats at the onset of the dark phase.

The dark phase is the rats' natural feeding time. Therefore, assessing the effect of a putative satiety factor in non-fasted animals at this time would represent a more physiological effect.

Effect of OXM on food intake.

In study 3a, when injected in the early dark phase, both GLP-1 and OXM (3 nmol) significantly reduced food intake compared with that of saline-treated animals 1h postinjection [Fig. 3A].

Observation of behaviour after ICV injection of OXM.

ICV administration of OXM (3 nmol) in the early dark phase led to a significant decrease in feeding episodes (study 3a) and an increase in rearing behaviour (study 3b) [Fig. 3B]. There was no change in grooming, still, head down, burrowing, or locomotion episodes.

To assess whether OXM acts via the GLP-1R, a study using the GLP-1R antagonist, exendin-(9-39) was performed.

ICV administration. Study 4.

- 5 ICV coadministration of the GLP-1 receptor antagonist exendin-(9-39) with GLP-1 at a ratio of 10:1 (antagonist/agonist) blocked the anorectic effects of GLP-1 [Fig. 4A]. Furthermore, coadministration of exendin-(9-39) with OXM resulted in attenuation of the anorectic effect of OXM [Fig 4A].

- 10 iPVN administration.

Similarly, when injected iPVN, the anorectic effects of both GLP-1 and OXM were blocked when coinjected with exendin-(9-39) [Fig 4B].

Receptor binding assays. Study 5.

- 15 The affinity ( $IC_{50}$ ) of GLP-1 for the GLP-receptor in rat hypothalamic membrane preparations was 0.16 nM (Fig. 5). The affinity of OXM for the GLP-1 receptor in the same membrane preparations was 8.2 nM (Fig. 5), which is approximately 2 orders of magnitude weaker than that of GLP-1.

- 20 Discussion.

- OXM causes a potent decrease in fasting-induced refeeding when injected both ICV and iPVN. The effect was sustained until 8h (iPVN) or 4h (ICV) postinjection. The effect of OXM is approximately of the same magnitude and time course as that of GLP-1 when administered ICV and iPVN at equimolar doses. In addition, OXM  
25 inhibits food intake in nonfasted rats at the onset of the dark phase, and at that time they showed no signs of aversive behaviour.

- It has been suggested that there is an OXM-specific binding site in gastric mucosa. However, no such binding site has been identified in the CNS. Therefore, it was  
30 proposed that OXM mediated its effects via the hypothalamic GLP-IR, as GLP-1 and OXM have similar potency in feeding studies. It has been shown that OXM has a nanomolar affinity for the GLP-IR ( $IC_{50} = 8.2$  nM). This affinity is approximately 2

orders of magnitude weaker than that of GLP-1 ( $IC_{50} = 0.16$  nM). Yet despite this reduced affinity for the GLP-1R, OXM reduces food intake to the same magnitude. One explanation for this is that OXM might act through both the GLP-1R and its own receptor in the hypothalamus. Thus, OXM could elicit a response comparable to that of GLP-1 despite its lower affinity for the GLP-1R.

Exendin-(9-39), a fragment of the GLP-1R agonist exendin-4, is a potent and selective antagonist at the GLP-1R. When GLP-1 and exendin-(9-39) are coinjected, the anorectic actions of GLP-1 are blocked. When OXM is coinjected with exendin-(9-39), the anorectic effects of OXM are also completely blocked. This would strengthen the argument that OXM is mediating its effects via the GLP-1R.

We investigated the effects of glicentin, and glucagon after an acute ICV injection in fasted rats. No effect on fasting-induced food intake was seen after the administration of these peptides. In addition, there was no effect of these peptides when they were administered iPVN. When SP-1, the putative minimal active structure of OXM, was injected iPVN, no inhibition of food intake was observed. Therefore the effect seen by OXM is specific.

## 20 Example 2

Peripheral administration of OXM also reduces food intake and body weight gain.

### Peptides and chemicals

OXM was purchased from IAF BioChem Pharma (Laval, Canada). GLP-1 was purchased from Peninsula Laboratories Inc. (St. Helens, UK). Exendin 9-39 was synthesised at Medical Research Council, Hemostasis Unit, Clinical Sciences Centre, Hammersmith Hospital, London, UK using F-moc chemistry on a 396 MPS peptide synthesizer (Advanced ChemTech Inc., Louisville, KY) and purified by reverse phase HPLC on a C<sub>8</sub> column (Phenomex, Macclesfield, UK), using a gradient of acetonitrile on 0.1 % trifluoroacetic acid. Correct molecular weight was confirmed by mass spectrometry. All chemicals were purchased from Merck Eurolab Ltd. (Lutterworth, Leicestershire, UK), unless otherwise stated.

### Animals

Adult male Wistar rats (180 – 200 g) were maintained in individual cages under controlled conditions of temperature (21-23 °C) and light (12 hours light, 12 hours dark) with ad libitum access to standard rat chow (RM1 diet, Special Diet Services UK Ltd., Witham, Essex, UK) and water. All procedures undertaken were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (Project Licenses PPL: 90/1077, 70/5281 and 70/5516).

### 10 Intra-arcuate nucleus cannulation

Animals had permanent indwelling, unilateral, stainless steel guide cannulae (Plastics One, Roanoke, VA) stereotactically implanted into the arcuate nucleus of the hypothalamus, using a cannulation protocol using cannulae positioned 3.3 mm posterior to and 0.3 mm lateral to bregma and 9.0 mm below the outer surface of the skull.

### Intra-peritoneal (IP) injections

All IP injections were delivered using a 1 ml syringe and a 25 gauge needle. The maximum volume of injection was 500 µl, and was adjusted according the weight of the individual animal. All peptides were dissolved in saline.

In these studies, the human OXM and human GLP-1 were used with the sequences provided on pages 15 and 16 above.

### 25 In vivo protocols

1. Investigating the dose-response effect of peripheral administration of OXM on food intake in fasted animals:

Animals were fasted for 24 hours prior to the study. During the early light phase (09.00 – 10.00 hr), rats were given a single IP injection of saline, GLP-1 (30 nmol/kg body weight as a positive control) or OXM (10 – 300 nmol/kg body weight) (n = 12 per group) in a volume of 500 µl. Following the injection, the animals were returned

to their home cages and provided with a pre-weighed amount of chow. Food intake was measured 1, 2, 4, 8 and 24 hours post-injection.

2. Investigating the effect of peripheral administration of OXM on food intake in non-  
5 fasted animals during the dark phase:

The dark phase is the “normal” feeding time for rats. Therefore, any inhibition of food intake at this time could be considered to be more physiological than alterations to refeeding following a fast. Animals received a single IP injection of saline or  
10 OXM (3 – 100 nmol/kg body weight) (n= 12 per group) prior to lights out (18.00 – 19.00 hr). Food intake was measured 1, 2, 4, 8 and 12 hours post-lights-out.

3. The effect of repeated IP injections of OXM

45 Animals were randomised by weight into three groups (n = 15 per group): 1) Saline-treated with ad libitum access to food, 2) OXM-treated (50 nmol/kg body  
15 weight per injection – a dose based on the previous dose-response experiment) with ad libitum access to food, 3) Saline-treated, but food restricted to the mean light and dark phase food intake of the OXM-treated group. Animals were injected twice daily (07.00 and 18.00 hr) for seven days. Food intake (g), body weight (g) and water  
20 intake (ml) were measured daily. On the eighth day, the animals were killed by decapitation. Epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were removed and weighed as an assessment of body adiposity.

4. Investigating the effect of peripheral administration of OXM on gastric emptying  
Animals were fasted for 36 hours to ensure that the stomach was empty. During the  
25 early light phase (09:00-10:00) were allowed ad libitum access to a pre-weighed amount of standard rat chow for thirty minutes. After that time, the food was removed and reweighed. The animals were then IP injected with saline, OXM (50 nmol/kg body weight) or CCK-8 (15 nmol/kg body weight). Rats were then killed at the same times as those used in the previous feeding studies: 1, 2, 4 or 8 hours post-  
30 feeding (n = 12 per group per time-point). The CCK-8 group was used as a positive control for the experiment at the two-hour time-point only. Animals were killed by carbon dioxide asphyxiation. A laparotomy was rapidly performed and the stomach

exposed. The pyloric junction was ligated (2.0 Mersilk, Johnson & Johnson, Belgium), followed by ligation of the gastro-oesophageal junction and the stomach was removed. The gastric contents were then removed, placed in a pre-weighed weighing boat and left to air-dry for 48 hours. Once dry, the contents were weighed and the percentage of the chow ingested during the half-hour re-feeding period remaining in the stomach per rat was then calculated using the following formula:

$$\% \text{ food remaining in the stomach} = \frac{\text{dry weight of stomach content}}{\text{weight of food ingested}} \times 100$$

10

5. Investigating the effect of increasing doses of intra-arcuate OXM

Intra-arcuate (Intra-ARC (iARC)) cannulated rats (n = 12-15 per group) were randomised by weight into 6 groups. During the early light phase (0900 – 1000), 24-hour fasted rats received an iARC injection of saline, OXM (0.01, 0.03, 0.1, 0.3 or 1.0 nmoles). Food intake was measured 1, 2, 4, 8 and 24 hours post-injection.

15

6. Investigating whether peripherally administered OXM is acting directly via arcuate nucleus GLP-1 receptors.

Rats cannulated into the arcuate nucleus were randomised into 6 groups (n = 10-12 per group). During the early light phase (0900 – 1000) 24-hour fasted rats received an iARC injection of saline or exendin<sub>9-39</sub> (5 nmoles) followed by an IP injection of saline, OXM (30 nmoles / kg body weight) or GLP-1 (30 nmoles / kg body weight) 15 minutes later. The injection details are described in Table 1 below.

20

25

| Group | Intra-ARC injection        | IP injection         |
|-------|----------------------------|----------------------|
| 1     | Saline                     | Saline               |
| 2     | Saline                     | OXM (30 nmoles/kg)   |
| 3     | Saline                     | GLP-1 (30 nmoles/kg) |
| 4     | Exendin 9-39<br>(5 nmoles) | Saline               |

|   |                            |                      |
|---|----------------------------|----------------------|
| 5 | Exendin 9-39<br>(5 nmoles) | OXM (30 nmoles/kg)   |
| 6 | Exendin 9-39<br>(5 nmoles) | GLP-1 (30 nmoles/kg) |

---

Table 1

## Immunohistochemistry

- 5 90 minutes after an IP injection of OXM (50 nmol/kg), CCK (15 nmol/kg) or saline, rats were terminally anaesthetized and transcardially perfused with 0.1 M phosphate buffered saline (PBS) following by 4 % PB-formalin (PBF). The brains were removed and post-fixed overnight in PBF and then transferred to PB-sucrose (20 % w/v) overnight. 40  $\mu$ m coronal sections of brain and brainstem were cut on a freezing
- 10 microtome and stained for fos-like immunoreactivity (FLI) by the avidin-biotin-peroxidase method. The sections were then mounted on poly-L-lysine-coated slides, dehydrated in increasing concentrations of ethanol (50 – 100 %), delipidated in xylene and coverslipped using DPX mountant. Slides were examined for FLI-positive nuclei using a light microscope (Nikon Eclipse E-800) and images captured using a
- 15 microimager (Xillix MicroImager). The numbers of FLI-positive nuclei in the hypothalamus and brainstem were counted by an independent member of the research team who was blinded to the experimental groups. The average number of FLI-positive nuclei per section was calculated and expressed as an integer for each animal.
- 20 Hypothalamic explant static incubation
- A static incubation system was used. Male Wistar rats were killed by decapitation and the whole brain removed immediately. The brain was mounted, ventral surface uppermost, and placed in a vibrating microtome (Microfield Scientific Ltd., Dartmouth, UK). A 1.7 mm slice was taken from the basal hypothalamus, blocked
- 25 lateral to the Circle of Willis and incubated in chambers containing 1 ml of artificial cerebrospinal fluid which was equilibrated with 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. The hypothalamic slice encompassed the medial pre-optic area, PVN (paraventricular hypothalamic nucleus), dorsomedial nucleus, ventromedial nucleus, lateral

hypothalamus and ARC. The tubes were placed on a platform in a water bath maintained at 37°C. After an initial 2-hour equilibration period, each explant was incubated for 45 minutes in 600 µl aCSF (basal period) before being challenged with a test period. OXM, 100 nM was used as a dose representing a concentration ten  
5 times that of its IC<sub>50</sub> for the GLP-1 receptor. The viability of the tissue was confirmed by a final 45-minute exposure to aCSF containing 56 mM KCl. At the end of each experimental period, the aCSF was removed and stored at -20 °C until measurement of αMSH-immunoreactivity by radioimmunoassay.

#### 10 Radioimmunoassay to measure αMSH-IR

Alpha-MSH was measured using an in-house radioimmunoassay, developed using an antibody from Chemicon International Inc.

#### Statistical analysis

Data from IP and iARC feeding studies were analyzed by ANOVA with post-hoc  
15 LSD (least significant difference) test. Fat pad weights from different treatment groups were analyzed using an unpaired t test. Data from the hypothalamic explant incubation study, in which each explant was compared with its own basal period, were analyzed by paired t test. In all cases P<0.05 was considered to be statistically significant.

#### 20

#### Results

##### 1. The effect of peripheral administration of OXM in fasted animals:

Intraperitoneal administration of OXM (100 nmol/kg and 300 nmol/kg) caused a significant inhibition in refeeding in 24-hour fasted animals one hour post-injection, compared with saline controls (1 hour: OXM 100 nmol/kg, 5.4 ± 0.2 g (P<0.05), 300  
25 nmol/kg, 4.5 ± 0.2 g (P<0.05) vs. saline, 6.3 ± 0.2 g). The reduction in food intake caused by 100 nmol/kg was sustained until 8 hours post-injection. However, the highest dose of OXM (300 nmol/kg) continued to significantly inhibited food intake 24 hours post-injection (8 hours: OXM, 300 nmol/kg, 9.5 ± 0.6 g vs. saline, 17.5 ± 0.7  
30 g; P<0.05) (Figure 6a). The 30 nmol/kg and 10 nmol/kg failed to alter food intake at any time-point investigated.

2. The effect of peripheral administration of OXM in non-fasted animals on dark phase food intake:

OXM, 3 and 10 nmol/kg, failed to affect food intake at any time-point investigated in nocturnally feeding rats injected immediately prior to the dark phase. However, OXM, 30 nmol/kg, significantly inhibited food intake until 2 hours post-injection (2 hours: OXM, 30 nmol/kg,  $4.5 \pm 0.4$  g vs. saline,  $5.8 \pm 0.4$  g;  $P < 0.05$ ). Food intake was reduced 4 hours post-injection, but this was not significant. OXM, 100 nmol/kg, significantly inhibited food intake throughout the dark phase (8 hours: OXM, 100 nmol/kg,  $14.1 \pm 0.8$  g vs. saline,  $16.9 \pm 0.5$  g;  $P < 0.05$ ) (Figure 6b).

3. The effect of repeated IP administration of OXM

Twice-daily IP injections of OXM (50 nmol/kg) for seven days caused a significant decrease in cumulative daily food intake, compare with saline-treated control animals (Cumulative food intake day 7: OXM, 50 nmol/kg,  $168 \pm 4.6$  g vs. saline,  $180 \pm 4.3$  g;  $P < 0.01$ ) (Figure 7a). Furthermore, OXM-treated animals gained weight significantly more slowly than saline controls (cumulative weight gain day 7: OXM, 50 nmol/kg,  $21.0 \pm 1.5$  g vs. saline,  $37.6 \pm 1.9$  g;  $P < 0.005$ ). Moreover, the food restricted “pair fed” animals did not gain weight as slowly as OXM-treated animals, despite receiving the same food intake (Day 7: pair fed,  $33.5 \pm 2.0$  g;  $P = \text{NS}$  vs. saline (ad libitum fed),  $P < 0.05$  vs. OXM) (Figure 7b). In addition, chronic OXM caused a decrease in adiposity that was not seen in saline-injected pair fed animals (Table 2). Water intake was significantly reduced in OXM-treated animals on days 1 and 2 of the experiment (Day 1: OXM,  $24.1 \pm 1.28$  ml vs. saline,  $28.1 \pm 1.33$  ml;  $P < 0.05$ ). On subsequent days, there was an increase in daily water intake compared with saline-treated animals (days 3-6). However, by day 7, there was no difference in water intake between saline and OXM-treated groups (not shown). The body weight difference between the “pair fed” rats and the OXM treated rats is due to increased energy expenditure since the two groups ate the same amount of food.

| <u>Tissue/hormone</u> | <u>Saline</u>                     | <u>OXM</u>                          | <u>Pairfed</u>                      |
|-----------------------|-----------------------------------|-------------------------------------|-------------------------------------|
| <u>WAT</u>            | <u><math>0.69 \pm 0.02</math></u> | <u><math>0.51 \pm 0.01^a</math></u> | <u><math>0.61 \pm 0.02^b</math></u> |
| <u>BAT</u>            | <u><math>0.16 \pm 0.01</math></u> | <u><math>0.12 \pm 0.01^a</math></u> | <u><math>0.15 \pm 0.01^b</math></u> |

Table 2: The effect of twice-daily IP administration of saline or OXM (50 nmol/kg) for seven days on the weight of epididymal WAT and interscapular BAT in food restricted and ad libitum fed rats.

5

#### 4. The role of delayed gastric emptying on the anorectic effect of OXM:

One hour after food was presented to the 36-hour fasted rats, the dry weight of the contents of the stomachs (as a percentage of the food consumed during the 30 minute feeding period) of GLP-1-treated animals were significantly greater than that of saline-treated animals (1 hour: GLP-1, 50 nmol/kg,  $76.9 \pm 2.7$  g vs. saline,  $65.8 \pm 1.6$  g;  $P < 0.01$ ), suggesting that GLP-1 caused a significant decrease in gastric emptying. The contents of the stomachs of OXM-treated animals were greater than those of the saline treated controls, although this was not statistically significant (1 hour: OXM, 50 nmol/kg,  $72.0 \pm 1.4$  g vs. saline  $65.8 \pm 1.6$  g;  $P = 0.07$ ). Two hours post-feed, OXM did not affect the contents of the stomach, compared with saline-treated animals. However, animals injected with the positive control for this time-point, CCK (15 nmol/kg), had significantly greater stomach content (2 hours: CCK, 15 nmol/kg,  $64.7 \pm 6.4$  g vs. saline, 38.5 g;  $P < 0.01$ ), suggesting that CCK caused a significant decrease in the rate of gastric emptying. There was no effect of OXM on the contents of the stomach, compared with saline-treated animals, at 4 or 8 hours post-feed (Figure 8).

5. Investigating the effect of increasing doses of OXM injected intra-arcuate nucleus Food intake was significantly inhibited by all doses (except 0.01 nmoles) of OXM administered iARC during the 1<sup>st</sup> hour of re-feeding following a 24-hour fast (1 hour: OXM 0.03 nmoles,  $6.1 \pm 0.5$  g ( $P < 0.05$ ); 0.1 nmoles,  $5.6 \pm 0.4$  g ( $P < 0.05$ ); 0.3 nmoles,  $5.1 \pm 0.6$  g ( $P < 0.01$ ); 1.0 nmole,  $3.6 \pm 0.5$  g ( $P < 0.005$ ) all vs. saline,  $7.7 \pm 0.2$  g) (Figure 9). OXM 0.3 and 1.0 nmoles continued to significantly inhibit food intake until 8 hours post-injection. Twenty-four hours post-injection, food intake was

inhibited by OXM 1.0 nmoles, although this was not significant (24 hours: OXM, 1.0 nmole,  $37.8 \pm 3.0$  g vs. saline,  $40.8 \pm 1.6$  g;  $P=NS$ ).

## 6. Investigating whether peripherally administered OXM is acting via arcuate nucleus 5 GLP-1 receptors

Intraperitoneal administration of both GLP-1 (30 nmol/kg) and OXM (30 nmol/kg) caused a significant inhibition of food intake one hour into the dark phase (1 hour: GLP-1,  $5.0 \pm 0.6$  g, OXM,  $5.1 \pm 0.4$  g vs. saline,  $9.2 \pm 0.3$  g). However, the anorexia caused by IP administration of OXM was blocked by prior administration of the GLP-1  
10 1 receptor antagonist, exendin 9-39 (300 nmol/kg), injected directly into the ARC (Table 3 & Figure 10). Inhibition of food intake by IP GLP-1 was not affected by prior iARC administration of exendin 9-39.

| Peptide               | Food intake (g) | S.E.M. |
|-----------------------|-----------------|--------|
| Saline / saline       | 9.2             | 0.3    |
| Saline / GLP-1        | 5.0             | 0.6    |
| Exendin 9-39 / GLP-1  | 5.0             | 0.3    |
| Saline / OXM          | 5.1             | 0.4    |
| Exendin 9-39 / OXM    | 9.4             | 0.4    |
| Exendin 9-39 / saline | 9.0             | 0.3    |

15 Table 3: The effect of iARC administration of exendin 9-39 (5 nmoles) or saline injected 15 minutes prior to IP administration of OXM (30 nmol/kg), GLP-1 (30 nmol/kg) or saline on 1 hour food intake (g). (S = saline, G = GLP-1 (30 nmol/kg), Ox = OXM (30 nmol/kg), Ex = exendin 9-39 (5 nmoles)).

20 7. Mapping the expression of FLI in the hypothalamus in response IP OXM:  
After IP OXM administration (50 nmol/kg) dense staining of FLI was found almost exclusively in the hypothalamic arcuate nucleus (Figure 11a). No other hypothalamic nuclei (PVN (paraventricular hypothalamic nucleus), DMH (dorsomedial hypothalamic nucleus), VMH (ventromedial hypothalamic nucleus)) demonstrated  
25 specific c-fos staining.

In the brainstem, IP CCK (15 nmol/kg) caused dense staining of FLI, most notably in the NTS (nucleus tractus solitarius) and the area postrema (Figure 6b). However, neither IP saline nor IP OXM (50 nmol/kg) caused a specific increase in c-fos  
5 expression in the same brainstem nuclei investigated (Figure 11b).

#### 8. Changes in alpha-MSH release from hypothalamic explants when incubated with OXM

Incubating OXM (100 nM) with hypothalamic explants caused a significant increase  
10 in the release of  $\alpha$ -MSH, compared with basal release ( $\alpha$ -MSH: OXM, 100 nM,  $4.1 \pm 0.6$  fmol/explant vs.  $2.6 \pm 0.5$  fmol/explant;  $P < 0.005$ ). Explant viability was assessed by incubation with 56 mM KCl, and viability was confirmed in  $>80\%$  of explants. Those explants that were not viable were excluded from the analysis.

#### 15 Discussion

Peripheral administration of OXM causes a reduction in food intake in rats. This was seen following a fast in the light phase and during the nocturnal feeding phase. The anorectic effect was potent and sustained for periods up to 24 hours. Twice-daily IP administration of OXM for seven days caused a reduction in daily food intake  
20 compared with those treated with saline, with no tachyphylaxis. Animals treated with OXM gained significantly less weight than pair fed animals, despite the two groups receiving identical daily caloric intake. Intraperitoneal administration of OXM did transiently reduce water intake although this was not sustained, suggesting that the reduction in the rate of body weight gain was not due to dehydration.

25

On conclusion of the chronic study, epididymal WAT and interscapular BAT were removed and weighed. It was found that there was a reduction in the weights of all fat pads in OXM-treated animals compared with pair-fed animals, despite identical food intake. Therefore it appears that peripheral OXM administration is also affecting other  
30 metabolic parameters.

A major contributor to satiety is delayed gastric emptying via vagally-mediated mechanism that leads to brainstem activation. Both GLP-1 and OXM are potent inhibitors of gastric emptying in rodents and humans and in the case of GLP-1, this is thought to be the dominant mechanism through which it promotes satiety. We  
5 hypothesized that OXM was acting in the same way, and that its effects on gastric emptying were the cause of sustained anorexia. However, although peripheral administration of OXM led to a slight delay in gastric emptying in the first hour after the re-introduction of food, this was non-significant and the effect was short-lived. This suggested that OXM does slow gastric emptying, but it is not likely to be  
10 responsible for the robust and sustained inhibition of food intake.

We report here that peripheral administration of OXM increases FLI in almost exclusively in the ARC. Furthermore, we found that incubating hypothalamic explant with OXM caused a significant increase in the release of the POMC (pro-  
15 opiomelanocortin)-derived product,  $\alpha$ MSH from hypothalamic explants. IP OXM did not affect the expression of FLI in the NTS and AP – areas known to be important in integrating vagally mediated information, further strengthening the notion that OXM is not acting via these pathways.

20 It is thought that nuclei in the brainstem are the primary site of GLP-1 action, and information is subsequently relayed to the hypothalamic PVN, where its anorectic effects are mediated. Direct injection of OXM into the ARC, even at very low doses caused a robust and sustained inhibition of food intake, further supporting the hypothesis that that the ARC is the site of the actions of OXM. Anorectic effects  
25 caused by peripheral administration of OXM were blocked by prior administration of exendin 9-39 into the ARC. Interestingly, however, the anorectic actions of peripherally administered GLP-1 were not. This finding strongly indicates that OXM is acting via GLP-1 receptors in the ARC. In addition, it has identified distinct pathways which mediate the actions of GLP-1 and OXM.

30

Taken together, these data demonstrate that OXM is potentially important in both long and short-term regulation of food intake and body weight maintenance. Rather

than reducing appetite via “traditional” satiety pathways, involving slowing of gastric emptying and activation of brainstem nuclei, circulating OXM is mediating its anorectic effects via direct interaction with the ARC, potentially by activating POMC (pro-opiomelanocortin) neurons within the nucleus. Therefore, OXM may be useful  
5 in the treatment or prevention of excess weight such as obesity in mammals, and further represents a novel target for the development of therapeutic agents in the treatment of excess weight such as obesity in mammals.

### Example 3

#### 10 Investigation of The Effect of OXM Infusion on Food Intake in Human Subjects

##### Methods

##### Study 1

The study design was a double-blind placebo-controlled crossover, see Figure 12. 13  
15 healthy volunteers (age  $27 \pm 2$  yrs; BMI  $25.3 \pm 0.7 \text{ kg }^{-2}$ ) received a 90 minute intravenous infusion of OXM ( $3.0 \text{ pmol/kg/min}$ ) and an infusion of saline  $\geq 1$  week apart, in random order. OXM was dissolved in saline containing haemaccel (5% by volume) to reduce adsorption to the syringe and tubing. Volunteers completed a food diary for three days prior to each infusion and for the subsequent 24 hours. Subjects  
20 were instructed to follow a similar diet on the days preceding each infusion. They consumed an identical meal (of their choice) on the night before each infusion and fasted from 9 pm.

On each study day intravenous cannulae were inserted bilaterally into arm veins, one  
25 for administration of the infusion, while the other was used for blood-sampling. Subjects were attached to a cardiac monitor and blood pressure was measured every 15 min. Blood samples were collected every 30 minutes into Lithium-Heparin tubes (LIP LTD, UK) containing 5,000 Kallikrein Inhibitor Units (0.2 ml) of aprotinin (Trasylol, Bayer) and stored on ice. Following centrifugation plasma was immediately  
30 separated and stored at  $-70^{\circ}\text{C}$  until analysis.

15 min before termination of the infusion, subjects were offered a buffet meal which was provided in excess so that all appetites could be satisfied and subjects would be unable to assess their own food intake. The choices consisted of chicken curry, plain boiled rice, fruit salad, and a variety of mini chocolate bars and fruit-flavoured sweets.

5 Water was freely available. Dietary intake was calculated by weighing food and water pre and postprandially.

Food intake for 24 hours following the buffet meal was recorded in food diaries and energy intake was calculated with the aid of the Dietplan program (Forestfield Software LTD, West Sussex, UK).

10 Every 30 min subjects completed visual analogue scales (VAS) rating hunger, satiety, fullness, prospective food consumption and nausea. These consisted of 100 mm scales with the text expressing the most positive and the negative rating anchored at each end.

## 15 Study 2

The same protocol was followed as for Study 1, except that the eight healthy fasting volunteers were administered OXM subcutaneously at doses from 100 nmol to 250 nmol (in normal saline) thirty minutes before the buffet.

## 20 Results

### Study 1

OXM infusion led to a significant fall in calories consumed at the buffet meal ( $192 \pm 59$  kcal;  $17.6 \pm 5.7\%$ ). 12/13 subjects showed a decrease in calories consumed with OXM infusion, see Figure 13. OXM infusion was associated with a significant fall in subjective hunger scores, see Figure 14 (VAS 'How hungry are you right now?' 60 min  $P < 0.05$ ). There were no adverse effects of OXM infusion. In particular there was no effect of OXM on feelings of sickness (nausea) (VAS 'How sick do you feel right now?' 75 min  $P = 0.8$ ). The effect appears to be rapid.

## 30 Study 2

The results obtained are shown in Table 4.

| Name | wt KG | BMI | nmol |         | Energy (Kcal) |        | Kcal  |      |
|------|-------|-----|------|---------|---------------|--------|-------|------|
|      |       |     | dose | dose/kg | saline        | sc oxm | Diff  | Dif% |
| 01.  | 89    | 27  | 100  | 1.12    | 1344          | 283    | -1061 | -79  |
| 02   | 95    | 28  | 100  | 1.05    | 1059          | 840    | - 219 | -21  |
| 03   | 89    | 28  | 150  | 1.69    | 917           | 731    | -186  | -20  |
| 04   | 91    | 30  | 150  | 1.65    | 530           | 467    | -63   | -12  |
| 05   | 92    | 31  | 150  | 1.63    | 381           | 283    | -98   | -26  |
| 06   | 87    | 29  | 200  | 2.29    | 922           | 667    | -255  | -28  |
| 07   | 113   | 36  | 250  | 2.21    | 966           | 875    | -91   | -9   |
| 08   | 106   | 36  | 250  | 2.36    | 910           | 742    | -168  | -18  |
| MEAN |       |     |      |         | 879           | 611    | -268  | -32  |
| SEM  |       |     |      |         | 106           | 84     | 116   | 12   |

## Discussion

The demonstration that parenteral administration of OXM to human subjects results in a decrease in calories consumed and a significant reduction in subjective sensations of hunger without undesirable side effects, in particular, feelings of sickness (nausea) is confirmation the utility of OXM in the treatment or prevention of excess weight such as obesity in mammals, and as a novel target for the development of therapeutic agents in the treatment of excess weight such as obesity in mammals.

### Example 4

Investigation of the plasma OXM-immunoreactivity (IR) and ghrelin-

immunoreactivity IR (ghrelin-IR) levels following IP administration of OXM.

### Methods

OXM or saline were administered to fasted rats to investigate the plasma OXM-IR and ghrelin-IR levels following IP OXM. Plasma OXM-IR levels were measured, using a previously described assay, which also measures enteroglucagon (i.e., N-terminally elongated OXM) (Ghatei MA, Uttenthal LO, Christofides ND, Bryant MG, Bloom SR 1983 J Clin Endocrinol Metab 57:488-495). The OXM-IR assay could detect changes of 10 pmol/L (95% confidence limit) with an intra-assay variation of 5.7 %. The ghrelin radioimmunoassay (English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JP 2002 J Clin Endocrinol Metab 87:2984) measured both octanoyl and des-octanoyl ghrelin (Total ghrelin). It did not cross-react with any known gastrointestinal or pancreatic peptide hormones and could detect changes of 10 pmol/L (95 % confidence limit) with an intra-assay variation of 9.5 %.

Rats (n=10 per group) were IP injected with OXM (30 nmoles/kg and 100 nmoles/kg) or saline at the beginning of the light phase. The rats were decapitated 30 and 90 minutes following the IP injection, trunk blood collected. All plasma was collected and frozen at -20 C until assayed for OXM-IR and ghrelin-IR. During the entire post-injection period the rats remained fasted. The time points and the doses of OXM were chosen by reference to previous feeding studies.

The release of gut hormones has been found to be influenced by the content of the diet, in particular the fat content. For this reason, a further three groups (n = 10) were investigated: *a*) Rats fasted overnight and killed at the beginning of the light phase, *b*) Rats fed high fat rat chow (45 % fat, Research Diets Inc.) overnight and decapitated at the beginning of the light phase, *c*) Rats fasted overnight and at lights-on, they were given *ad libitum* access to 45 % high fat chow for 2 h. The rats were decapitated at the end of this 2-hour high fat meal (i.e., two hours into the light phase). All plasma was collected and frozen at -20°C until assayed for OXM-IR and ghrelin-IR.

## Results

IP administration of OXM (30 nmoles/kg and 100 nmoles/kg) increased plasma OXM-IR 30 and 90 minutes post-injection (30 min plasma OXM-IR pmol/L: saline  $61.8 \pm 8.9$ , OXM 30 nmoles/kg  $448.9 \pm 184.4$ , OXM 100 nmoles/kg  $997.1 \pm 235.4$ ).

90-min plasma OXM-IR pmol/L: saline  $47.5 \pm 4.5$ , OXM 30 nmoles/kg  $150.6 \pm 52.5$ , OXM 100 nmoles/kg  $107.8 \pm 25.0$ ).

The plasma OXM-IR levels were determined in three additional groups: *a*) Rats fasted overnight and killed at the beginning of the light phase (plasma OXM-IR pmol/L:  $51.9 \pm 5.8$ ), *b*) Rats fed high fat rat chow overnight and decapitated at the beginning of the light phase (plasma OXM-IR pmol/L:  $220.2 \pm 22.2$ ), *c*) Rats fasted overnight, then given *ad libitum* access to high fat chow for 2 hours at lights-on, were decapitated at the end of the 2-hour high fat meal (plasma OXM-IR pmol/L:  $254.0 \pm 32.7$ ).

IP administration of OXM (30 nmoles/kg and 100 nmoles/kg) significantly decreased fasting plasma ghrelin-IR 30 and 90 minutes post-injection (30 min plasma ghrelin pmol/L: saline,  $1056.9 \pm 64.0$ , OXM, 30 nmoles/kg  $867.4 \pm 42.0$  ( $p < 0.01$ ), OXM, 100 nmoles/kg  $860.0 \pm 47.5$  ( $p < 0.02$ ). Ninety-minute plasma ghrelin-IR pmol/L: saline,  $1055.2 \pm 52.5$ , OXM, 30 nmoles/kg  $886.9 \pm 36.3$  ( $p < 0.01$ ), OXM, 100 nmoles/kg  $900.0 \pm 52.9$  ( $P < 0.05$ ), see Figure 15.

Plasma ghrelin-IR levels were determined in 3 additional groups: *a*) Rats fasted overnight and killed at the beginning of the light phase (plasma ghrelin-IR pmol/L:  $1066.1 \pm 80.9$ ), *b*) Rats fed high fat rat chow overnight and decapitated at the beginning of the light phase (plasma ghrelin-IR pmol/L:  $611.3 \pm 16.9$ ), *c*) Rats fasted overnight, at lights-on they were given *ad libitum* access to high fat chow for 2 h, were decapitated at the end of the 2-hour high fat meal (plasma ghrelin pmol/L:  $648.9 \pm 57.3$ ).

### Example 5

#### Investigation of The Effect of OXM Infusion on Human Subjects

#### 30 Methods

##### Study design

The study design was as in Example 3. Subjects remained in the study room until  $t_{225}$ . They continued to complete VAS until 09:00 the following morning and recorded food intake in diaries for 24 hours following the buffet meal (until 13:00 the following day). Food diaries were analysed by a dietician blinded to the study and energy intake  
5 was calculated with the aid of the Dietplan program (Forestfield Software LTD, West Sussex, UK).

#### Plasma hormone measurements

All samples were assayed in duplicate and within one assay to eliminate inter-assay  
10 variation. Plasma OLI, pancreatic glucagon, peptide YY (PYY), insulin, glucagon-like peptide-1 (GLP-1) and ghrelin were measured using established in-house RIAs. The OLI assay (Ghatei MA, Uttenthal LO, Christofides ND, Bryant MG, Bloom SR 1983 J Clin Endocrinol Metab 57:488-495) could detect changes of 10 pmol/L (95% confidence limit) with an intra-assay variation of 5.7 %. The PYY assay (Adrian TE,  
15 Savage AP, Sagor GR, Allen JM, Bacarese-Hamilton AJ, Tatemoto K, Polak JM, Bloom SR 1985 Gastroenterology 89:494-499) could detect changes of 2 pmol/L (95 % confidence limit) with an intra-assay variation of 5.8 %. The PYY antibody was specific for the C-terminus of PYY and cross-reacts fully with human PYY 3-36. The insulin assay (Kreymann B, Williams G, Ghatei MA, Bloom SR 1987 Lancet  
20 2:1300-1304) could detect changes of 6 pmol/L (95 % confidence limit) with an intra-assay variation of 5.4 %. The GLP-1 assay (Kreymann B, Williams G, Ghatei MA, Bloom SR 1987 Lancet 2:1300-1304) could detect changes of 8 pmol/L (95 % confidence limit) with an intra-assay variation of 6.1 %. The GLP-1 antibody was specific for amidated GLP-1 and does not cross-react with GLP-1(1-37), GLP-1(1-36)  
25 or GLP-1(7-37). The ghrelin assay (English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JP 2002 J Clin Endocrinol Metab 87:298) could detect changes of 10 pmol/L (95 % confidence limit) with an intra-assay variation of 9.5 %. Plasma leptin was measured using the Linco Research (Missouri, USA) human leptin RIA kit.

#### Results

30 1. Effects of OXM infusion on energy intake

OXM infusion significantly reduced energy intake at the buffet meal by  $19.3 \pm 5.6 \%$  (reduction vs saline:  $220 \pm 60$  kcal,  $P < 0.01$ ). 12 out of the 13 subjects studied showed a decrease in energy intake with OXM infusion (Figure 17). There was no obvious cause for the failure of response in one subject. OXM infusion significantly  
5 reduced cumulative 12 hour energy intake by  $11.3 \pm 6.2 \%$  (reduction vs saline:  $365 \pm 159$  kcal,  $P < 0.05$ ) (figure 3). Cumulative 24 hour energy intake was not significantly altered (saline  $3043 \pm 366$  kcal, OXM  $2768 \pm 297$  kcal). OXM did not change water consumption or the proportion of calories obtained from different macronutrients at the buffet meal or in the subsequent cumulative 12 and 24 hr food intake.

10

## 2. Effects of OXM infusion on appetite and palatability

During infusion of saline visual analogue scores for hunger did not change significantly throughout the fasting period (Figure 18) whereas OXM infusion caused a significant fall in hunger (incremental AUC  $t_0$  to  $t_{75}$ : saline  $+273 \pm 128$  mm.min,  
15 OXM minus  $374 \pm 185$  mm.min,  $P < 0.05$ ). The decrease in hunger following the buffet meal was similar on saline and OXM infusion days and hunger scores remained similar thereafter. The duration of the meal was significantly reduced by OXM (saline  $19.2 \pm 1.3$  min, OXM  $15.1 \pm 1.8$  min,  $P < 0.05$ ). There was no significant effect of OXM on visual analogue scores for satiety, prospective food consumption, nausea  
20 and meal palatability (data not shown).

## 3. Plasma levels of OXM-like immunoreactivity (OLI)

Infusion of OXM elevated plasma OLI from  $62 \pm 5$  pmol/L to a peak of  $907 \pm 32$  pmol/L at  $t_{60}$  (Figure 19). In comparison, on the saline infusion day, consumption of  
25 the buffet meal led to a peak postprandial OLI level of  $151 \pm 18$  pmol/L at 195 min. Gel permeation analysis of plasma samples during OXM infusion (Figure 20) demonstrated a single immunoreactive peak eluting in the same position as synthetic OXM ( $K_{av} = 0.6$ ). Thus intact full-length OXM was the principle circulating form.

## 30 4. Effects of OXM infusion on plasma ghrelin

During the saline infusion, plasma ghrelin levels increased throughout the fasting period ( $t_0$   $461 \pm 32$  pmol/L,  $t_{75}$   $484 \pm 35$  pmol/L) and decreased postprandially ( $t_{225}$   $357 \pm 28$  pmol/L). However, during infusion of OXM fasting levels of ghrelin decreased before the meal ( $t_0$   $482 \pm 33$  pmol/L,  $t_{75}$   $435 \pm 35$  pmol/L) and there was a further postprandial reduction in ghrelin ( $t_{225}$   $356 \pm 31$  pmol/L). Hence plasma ghrelin prior to the buffet meal was significantly reduced by OXM infusion compared to saline (mean change in ghrelin from  $t_0$  to  $t_{75}$ : saline  $+24 \pm 10$  pmol/L, OXM minus  $47 \pm 11$  pmol/L,  $P < 0.0001$ ) (Figure 21). The suppression in plasma ghrelin due to OXM infusion represents  $44 \pm 10$  % of the postprandial decrease in ghrelin on the corresponding saline infusion day (mean postprandial decrease  $155 \pm 19$  pmol/L).

#### 5. Effects of OXM infusion on plasma hormones levels

There was no significant effect of OXM infusion on fasting plasma levels of PYY, insulin, pancreatic glucagon, GLP-1 or leptin (table 1). Plasma concentrations of leptin in female subjects were higher than in males as has previously been reported.

#### Discussion

We have demonstrated that systemic administration of OXM significantly reduces food intake in healthy human subjects. Intravenous infusion of OXM reduced calorie intake by 19 % at the buffet meal and cumulative energy intake was decreased in the 12 hours post-infusion. Much smaller alterations in food consumption would lead to weight loss if sustained in the long term. However there was no significant effect of OXM on cumulative 24 hour energy intake. Our work indicates that OXM may increase energy expenditure. OXM did not affect enjoyment of the meal, which is important in view of its potential therapeutic use.

Ghrelin is a powerful stimulant of appetite in man (Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR 2001 Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 86:5992) and preprandial rises in plasma ghrelin have been suggested to be a trigger for meal initiation (Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE,

Weigle DS 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50:1714-1719). Hence our novel finding that OXM infusion suppresses fasting plasma ghrelin is potentially important. Inhibition of the normal preprandial rise in ghrelin by OXM is likely to be one mechanism by which

5 OXM infusion reduces appetite. This finding may also shed light on the poorly understood mechanism by which ghrelin levels are reduced postprandially. In rodents, fasting increases plasma ghrelin, while oral intake of glucose, but not water, decreases ghrelin secretion, suggesting that suppression of plasma ghrelin is related to ingestion of nutrients rather than stomach distension.. Hence OXM released in response to

10 nutrient ingestion may contribute to the normal postprandial inhibition of plasma ghrelin. It is believed that only a proportion of total circulating ghrelin is the biologically-active, octanoylated form. The effect of food consumption and OXM infusion may be to primarily reduce levels of this active ghrelin.

15 Intravenous infusion of OXM has been shown to inhibit gastric emptying in humans. Suppression of gastric-emptying may lead to increased gastric distension which may contribute to satiety by causing a sensation of fullness. In the current study hunger scores were significantly reduced by OXM in the fasting state when gastric distension is unlikely to be important. Hence the reduction in appetite in the pre-meal period is

20 unlikely to result from effects of OXM on gastric emptying. The anorectic effect of OXM does not appear to be mediated by stimulation of the release of PYY or leptin as concentrations of these hormones were unaffected by OXM infusion.

We have demonstrated in humans the anorectic effect of elevated circulating levels of

25 OXM. Infusion of OXM produced circulating levels of OLI which were comparable to the elevated concentrations seen in tropical sprue and following jejuno-ileal bypass surgery for morbid obesity. Therefore OXM may contribute to the loss of appetite and weight loss observed in these conditions. We consider that lower postprandial concentrations of OXM contribute to the physiological reduction of appetite in normal

30 individuals and that exogenous administration of OXM has potential to reduce food intake and/or increase energy expenditure in the obese.

Taken together, these data demonstrate that OXM is potentially important in both long and short-term regulation of food intake, energy expenditure and body weight maintenance. Therefore, OXM may be useful in the treatment or prevention of excess weight such as obesity in mammals, and further represents a target for the development of therapeutic agents in the treatment of excess weight such as obesity in mammals, especially humans.

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## 9250wo sequence listing

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**CLAIMS:**

1. A method for decreasing calorie intake in a subject, a method for decreasing appetite in a subject, a method for decreasing food intake in a subject, a method for increasing energy expenditure in a subject, a method for weight control or treatment in a subject, or a method for reduction or prevention of obesity in a subject, which comprises administering oxyntomodulin to the subject.
2. A method for preventing and reducing weight gain in a subject; a method for inducing and promoting weight loss in a subject; or a method for reducing obesity as measured by the Body Mass Index, which comprises administering oxyntomodulin to the subject.
3. A method for controlling of any one or more of appetite, satiety and hunger in a subject, which comprises administering oxyntomodulin to the subject.
4. A method as claimed in claim 3 for inducing, increasing, enhancing or promoting satiety and/or sensations of satiety in a subject, which comprises administering oxyntomodulin to the subject.
5. A method as claimed in claim 3 for reducing, inhibiting or suppressing hunger or sensations of hunger in a subject, which comprises administering oxyntomodulin to the subject.
6. A method for maintaining desired body weight, a desired Body Mass Index, and/or a desired appearance and good health in a subject, which comprises administering oxyntomodulin to the subject.
7. A method for improving lipid profile in a subject, which comprises administering oxyntomodulin to the subject.

8. A method for alleviating a condition or disorder in a subject, which condition or disorder can be alleviated by reducing nutrient availability and/or by increasing energy expenditure, which comprises administering oxyntomodulin to the subject.
9. A method for reducing levels of circulating ghrelin in a subject, which comprises administering oxyntomodulin to the subject.
10. A method as claimed in any one of claims 1 to 9, wherein the effect is achieved by reducing levels of circulating ghrelin.
11. A method as claimed in any one of claims 1 to 10, wherein the oxyntomodulin is administered via a route peripheral to the brain.
12. A method as claimed in claim 11, wherein the oxyntomodulin is administered by an oral, mucosal e.g. buccal, sublingual, nasal, rectal, subcutaneous, transdermal intravenous, intramuscular or intraperitoneal route.
13. A method as claimed in any one of claims 1 to 12, wherein the oxyntomodulin is administered peripherally at a dose of, for example, 0.1 nmoles or more per kg body weight of the subject, for example, 0.2 nmoles or more, for example, 0.5 nmoles or more, for example, 1 nmole or more, for example, 1.5 nmoles or more, for example, 2 nmole or more, for example, 2.5 nmoles or more, for example, 3 nmoles or more, for example, 4 nmoles or more, for example, 5 nmoles or more, for example, 6 nmoles or more, for example, 7 nmoles or more, for example, 8 nmoles or more, for example, 9 nmoles or more, for example, 10 nmoles, for example, 11 nmoles or more, for example, up to 12 nmoles per kg body weight.
14. A method as claimed in any one of claims 1 to 12, wherein the oxyntomodulin is administered at a dose of up to 11 nmoles per kg body weight, for example, up to 10 nmoles, for example, up to 9 nmoles, for example, up to 8 nmoles, for example, up to 7 nmoles, for example, up to 6 nmoles, for example, up to 5 nmoles, for example, up to 4 nmoles, for example, up to 3 nmoles, for example, up to 2 nmoles, for

example, up to 1 nmoles, for example, up to 0.5 nmoles, for example, up to 0.4 nmoles, for example, up to 0.2 nmoles per kg body weight.

15. A method as claimed in any one of claims 1 to 14, wherein the oxyntomodulin is administered at a dose of 0.5mg to 2mg before meals.

16. A method as claimed in any one of claims 1 to 15, which comprises administering oxyntomodulin and one or more other agent(s), each of which has an influence in on weight and/or food intake.

17. A method as claimed in claim 16, wherein the other agent(s) each has any one of more of the following effects: reduces food intake and/or reduces hunger, reduces weight, reduces or prevents obesity, increases energy expenditure or reduces nutrient availability in a mammal.

18. A method as claimed in claim 16 or claim 17, where the other agent or one of the other agents is GLP-1 or an agonist thereof.

19. A method as claimed in claim 16 or claim 17, wherein the other agent or one of the other agents is PYY or an agonist thereof.

20. A method as claimed in claim 16 or claim 17, wherein the other agents are PYY or an agonist thereof and GLP-1 or an agonist thereof.

21. A method as claimed in any one of claims 16 to 20, wherein the oxyntomodulin and the other agent(s) are administered simultaneously, or sequentially in any order.

22. A method as claimed in any one of claims 16 to 21, wherein the PYY or agonist thereof and/or the GLP-1 or agonist thereof is administered peripherally at a dose of 0.1 nmoles per kg body weight of the subject or more, for example, 0.2 nmoles or more, for example, 0.4 nmoles or more, for example, 0.6 nmoles or more,

for example, 0.8 nmoles or more, for example, 1.0 nmole or more, for example, 1.2 nmoles or more, for example, 1.4 nmoles or more, for example, 1.6 nmoles or more, for example, 1.8 nmoles or more, for example, 2.0 nmoles or more, for example, 2.2 nmoles or more, for example, 2.4 nmoles or more, for example, 2.6 nmoles or more, for example, 2.8 nmoles, for example, 3.0 nmoles or more, for example, up to 3.2 nmoles per kg body weight.

23. A method as claimed in any one of claims 16 to 22, wherein the PYY or agonist thereof and/or the GLP-1 or agonist thereof is administered peripherally in an amount of up to 3.0 nmoles per kg body weight, for example, up to 2.8 nmoles, for example, up to 2.6 nmoles, for example, up to 2.4 nmoles, for example, up to 2.2 nmoles, for example, up to 2.0 nmoles, for example, up to 1.8 nmoles, for example, up to 1.4 nmoles, for example, up to 1.2 nmoles, for example, up to 1.0 nmoles, for example, up to 0.8 nmoles, for example, up to 0.6 nmoles, for example, up to 0.4 nmoles, for example, up to 0.2 nmoles per kg body weight.

24. Oxyntomodulin for use in a method as defined in any one of claims 1 to 23.

25. Use of oxyntomodulin for the manufacture of a medicament for use in a method as defined in any one of claims 1 to 23.

26. A pharmaceutical composition in unit dosage form comprising oxyntomodulin, in admixture or conjunction with a pharmaceutically suitable carrier, wherein the dose of oxyntomodulin is calculated on the basis of the per kg dose defined in claim 9 or claim 10.

27. A pharmaceutical composition in a form suitable for subcutaneous administration, which comprises from 0.5mg to 2 mg of oxyntomodulin per dose.

28. A pharmaceutical composition comprising oxyntomodulin and one or more other agent(s), each of which has an influence in on weight and/or food intake.

## 56

29. A pharmaceutical composition as claimed in claim 28, wherein the other agent(s) has any one of more of the following effects: reduces food intake and/or reduces hunger, reduces weight, reduces or prevents obesity, increases energy expenditure or reduces nutrient availability in a mammal.
30. A composition as claimed in claim 28 or claim 29, where the other agent or one of the other agents is GLP-1 or an agonist thereof.
31. A composition as claimed in claim 28 or claim 29, wherein the other agent or one of the other agents is PYY or an agonist thereof.
32. A composition as claimed in claim 28 or claim 29, wherein the other agents are PYY or an agonist thereof and GLP-1 or an agonist thereof.
33. A composition as claimed in any one of claims 30 to 32, in unit dosage form, wherein the dose of PYY and/or GLP-1 is as defined in claim 22 or claim 23, calculated on the basis of a 70 to 75 kg subject.
34. A composition as claimed in any one of claims 28 to 33, in a form suitable for administration via a route peripheral to the brain.
35. A composition as claimed in claim 34, in a form suitable for administration by an oral, mucosal e.g. buccal, sublingual, nasal, rectal, subcutaneous, transdermal intravenous, intramuscular or intraperitoneal route.
36. A composition as claimed in any one of claims 28 to 35, in unit dosage form, wherein the dose of oxyntomodulin is as defined in 10 or claim 11, calculated on the basis of a 70 to 75 kg subject.
37. A composition as claimed in claim 36, in a form suitable for subcutaneous administration, wherein the dose of oxyntomodulin is from 0.5mg to 2mg.

Preproglucagon

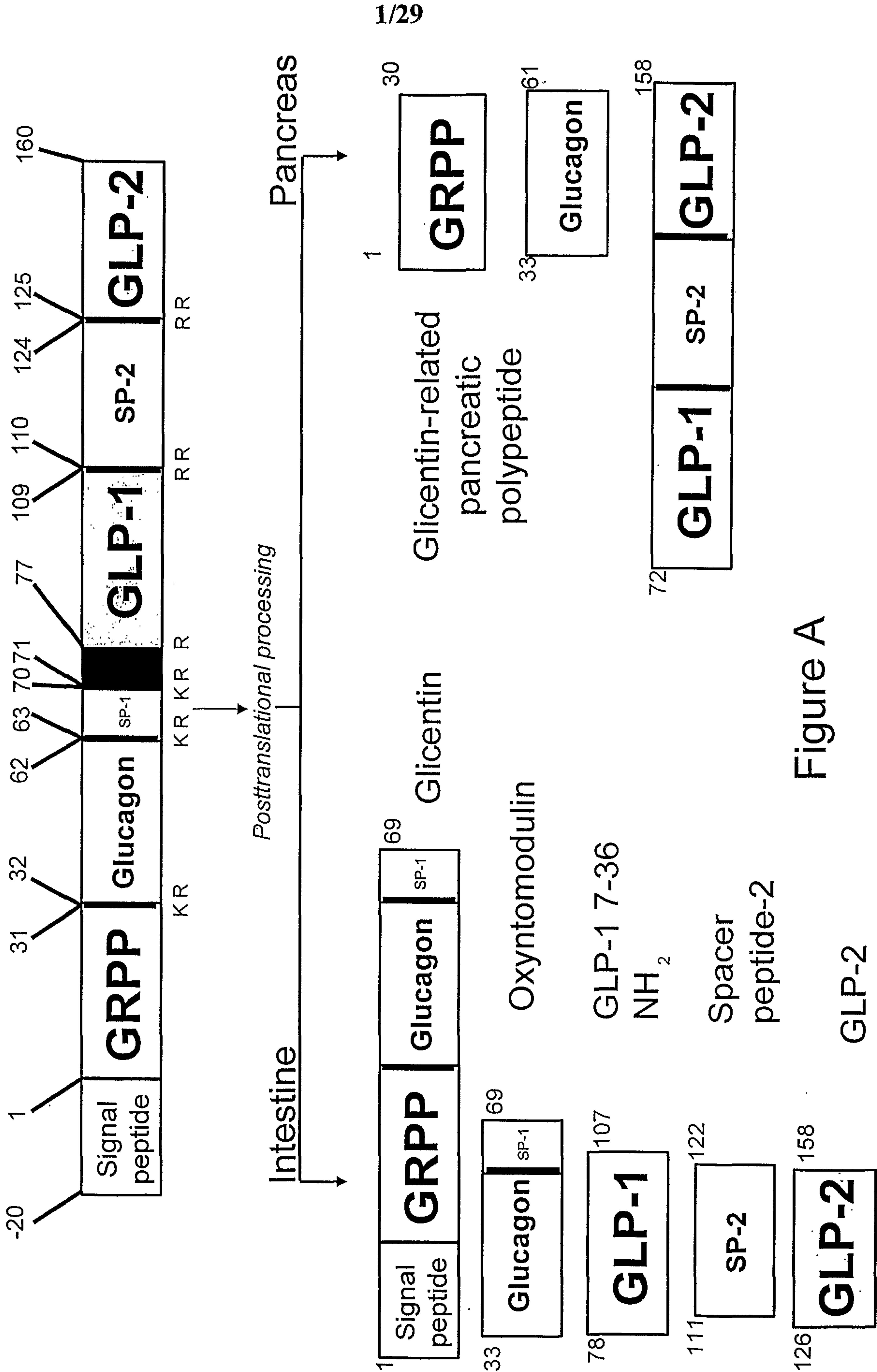


Figure A

2/29

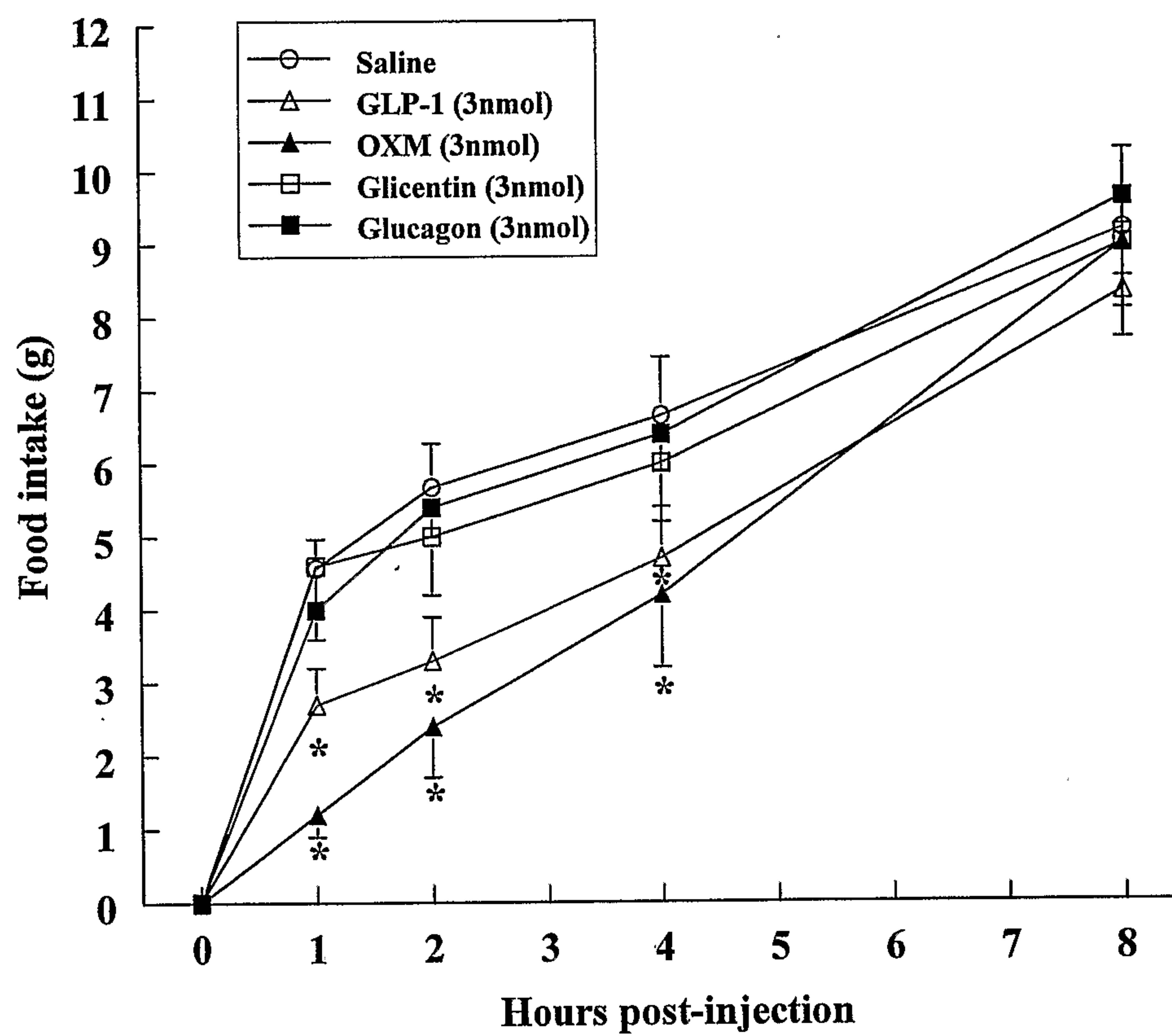


Figure 1A

3/29

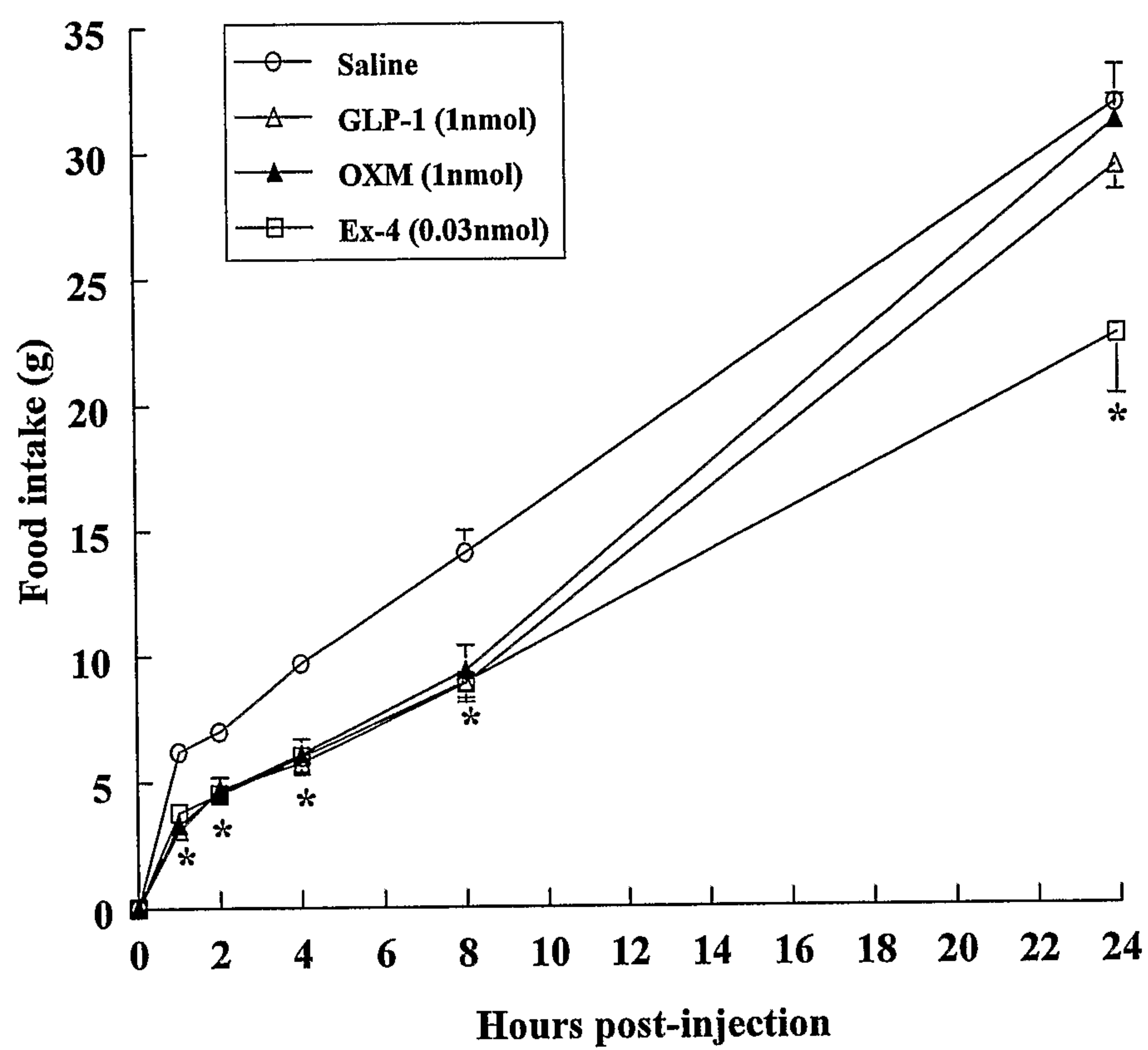


Figure 1B

4/29

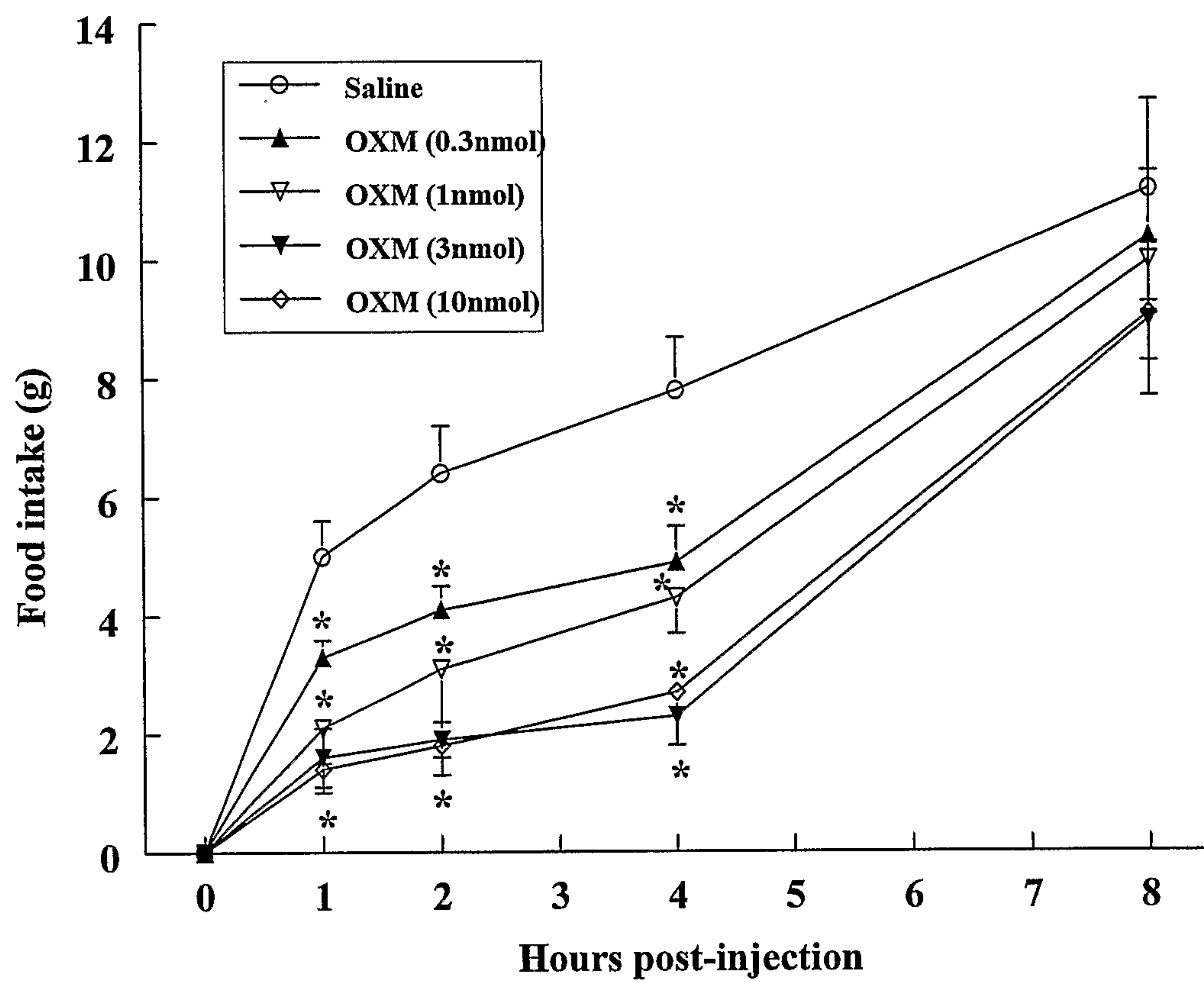


Figure 2A

5/29

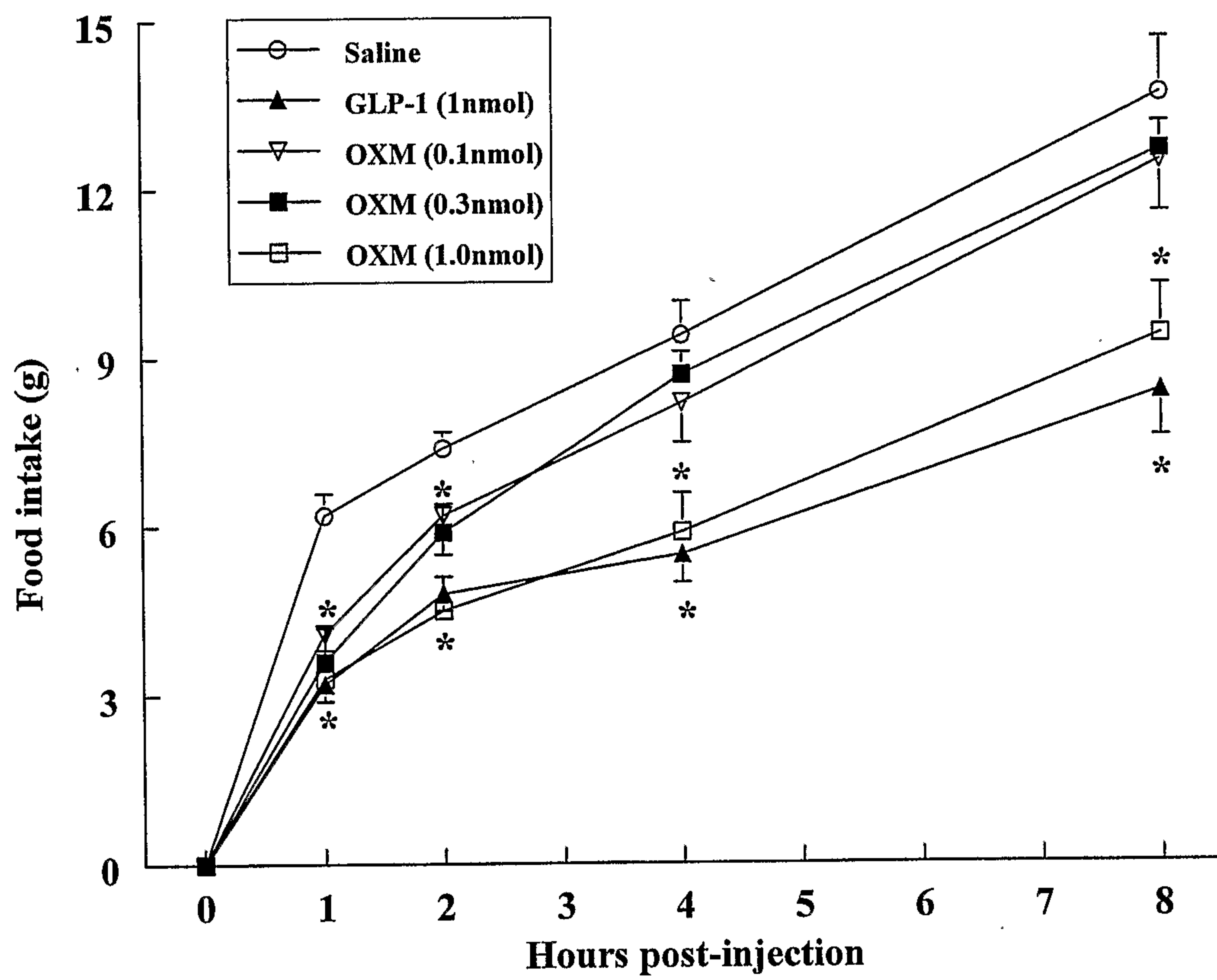


Figure 2B

6/29

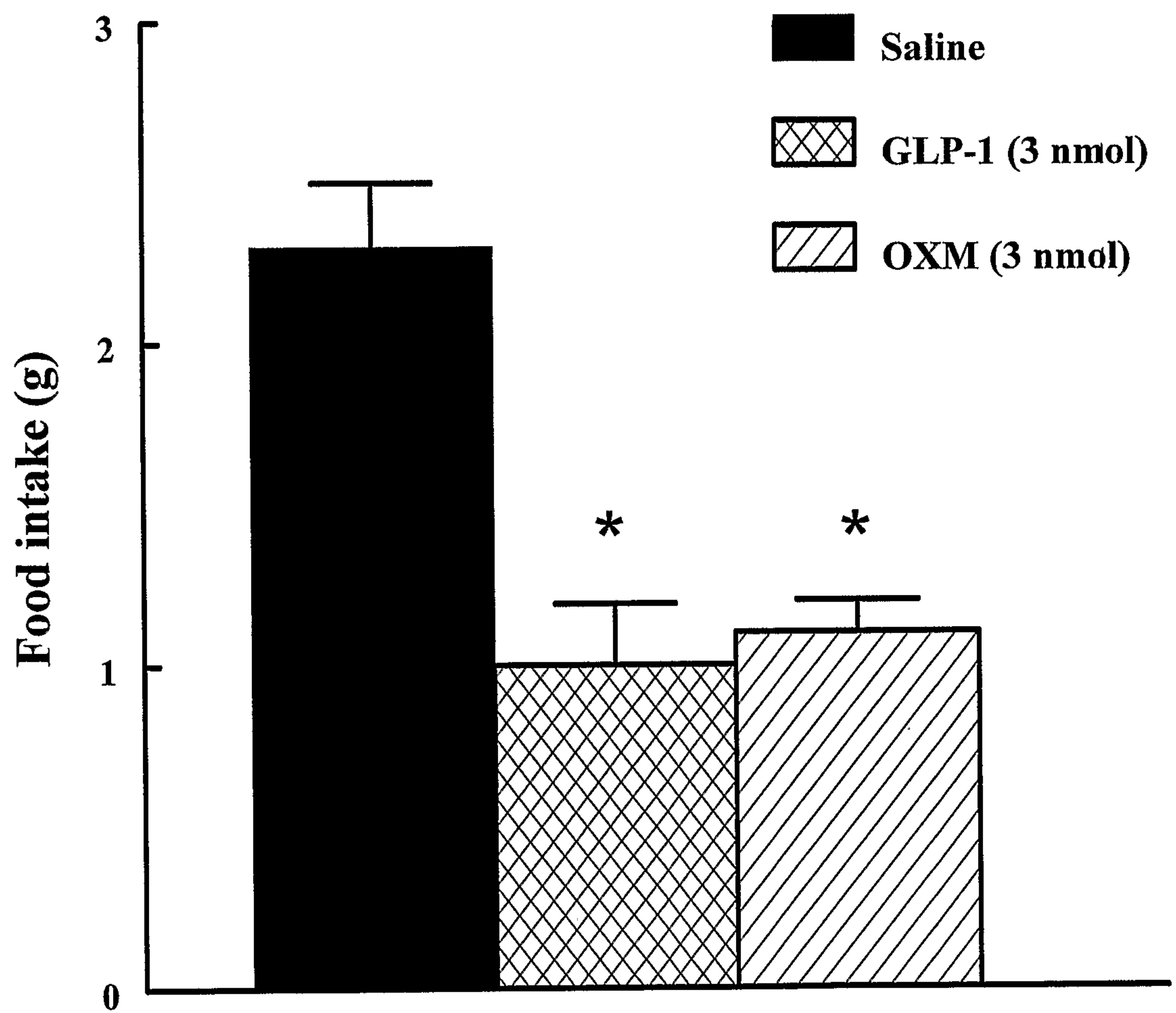


Figure 3A

7/29

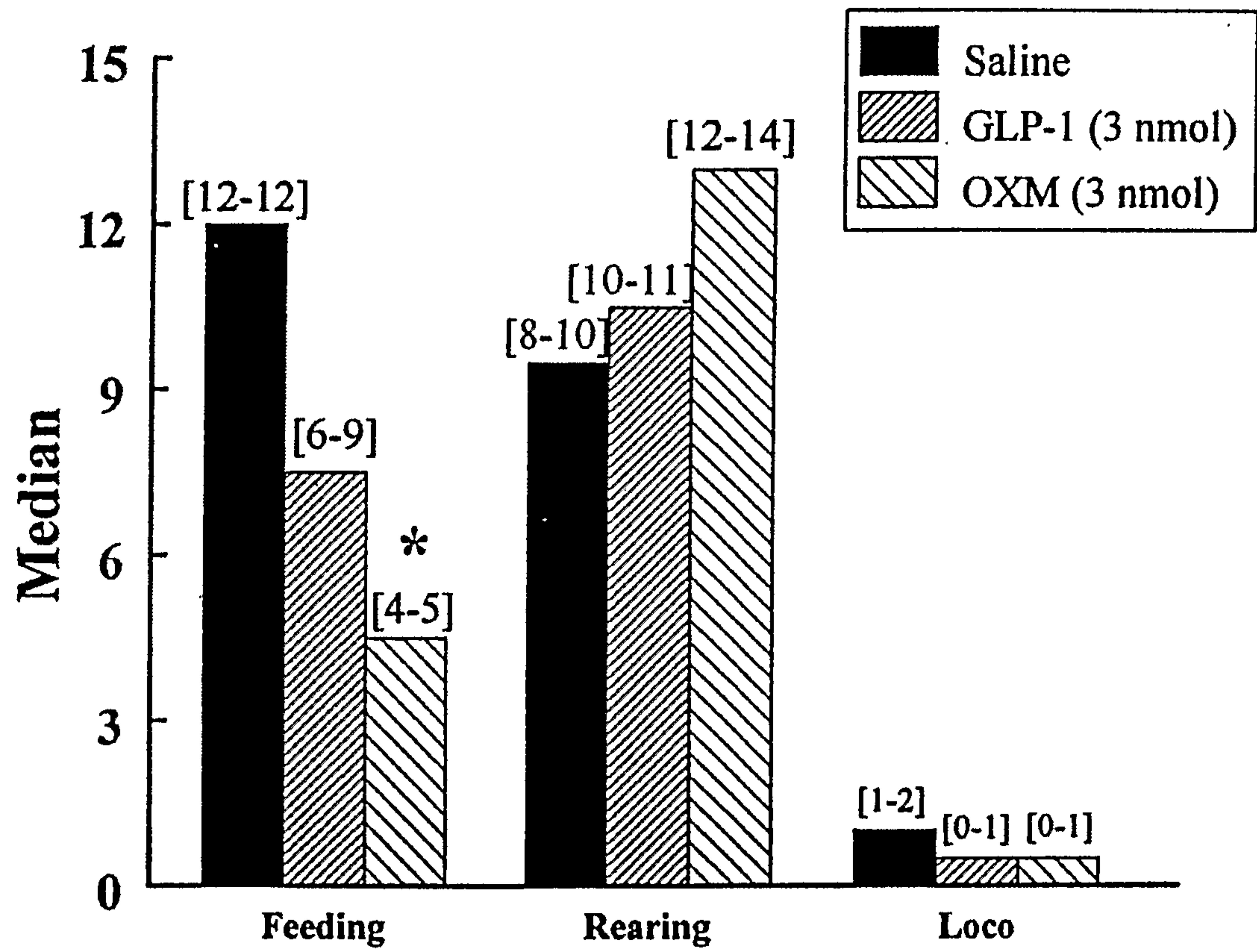


Figure 3B

8/29

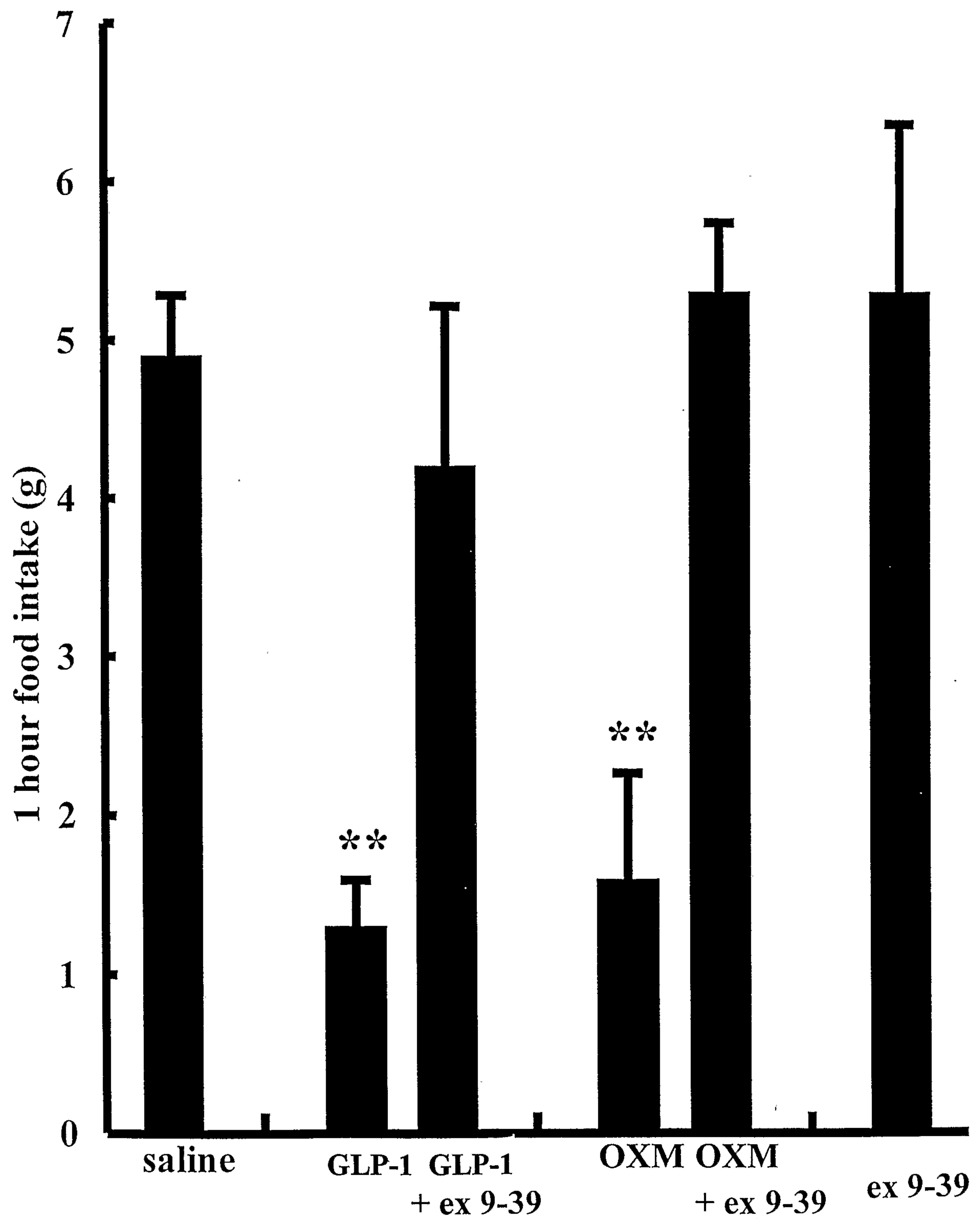


Figure 4A

9/29

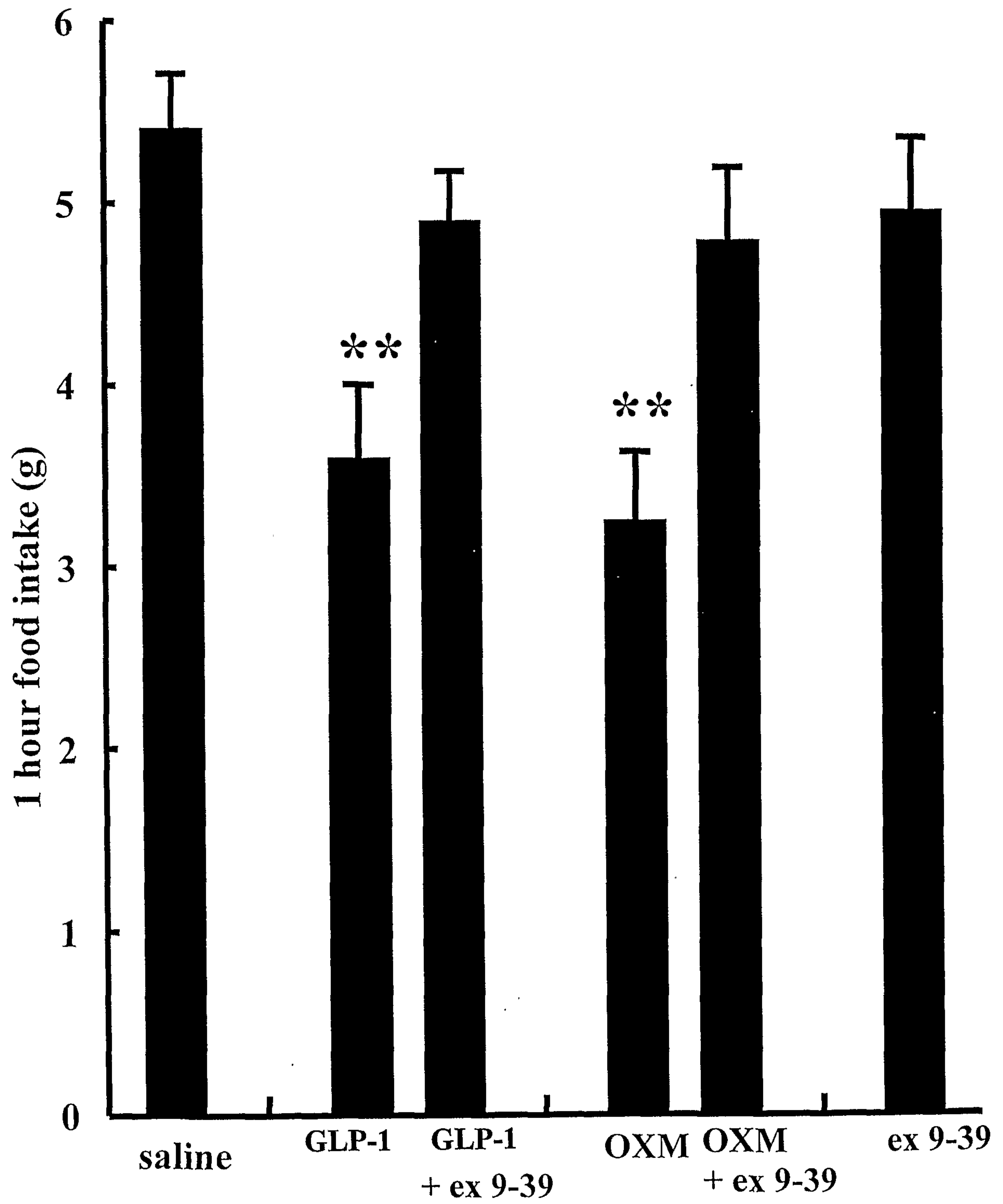


Figure 4B

10/29

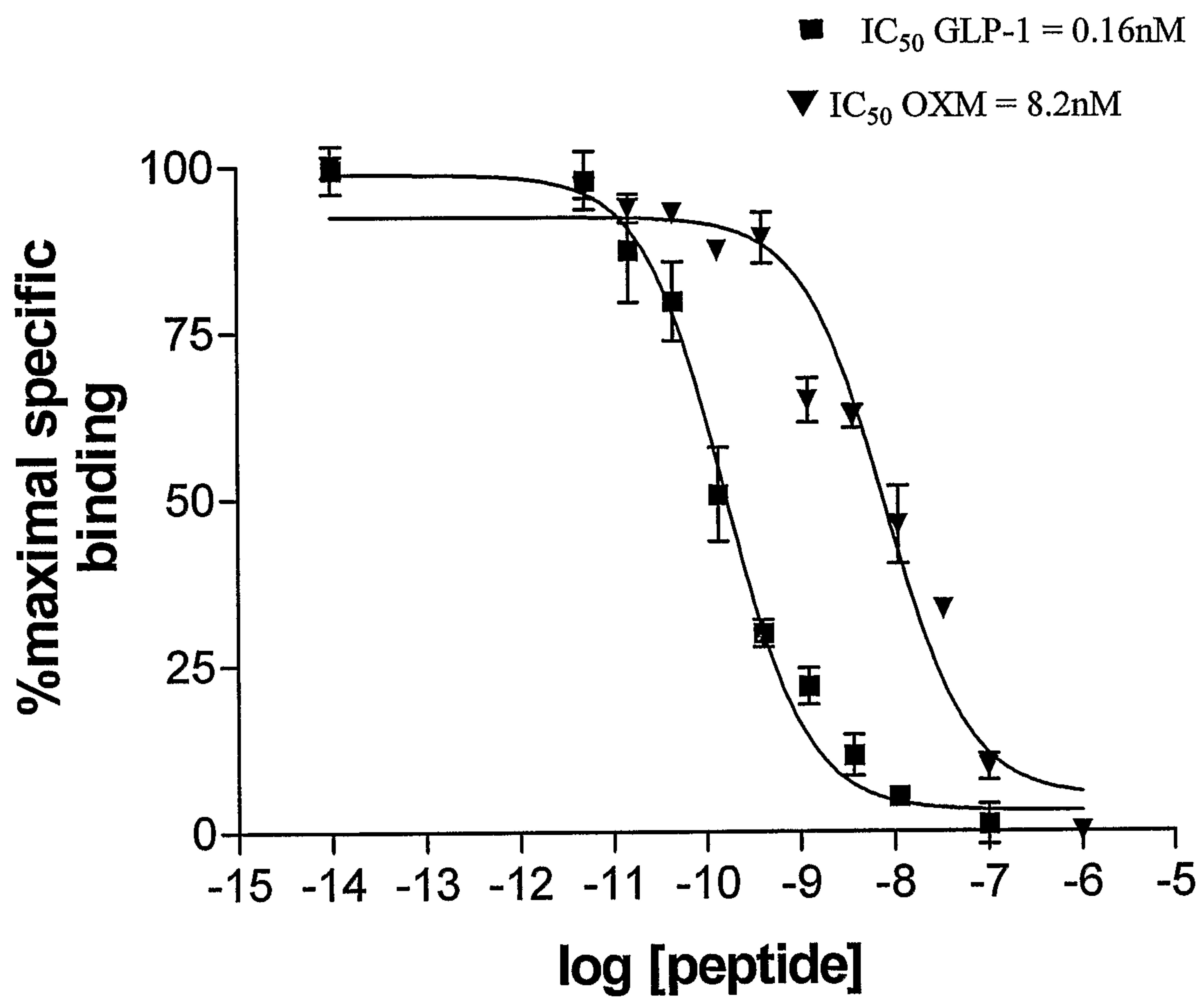


Figure 5

11/29

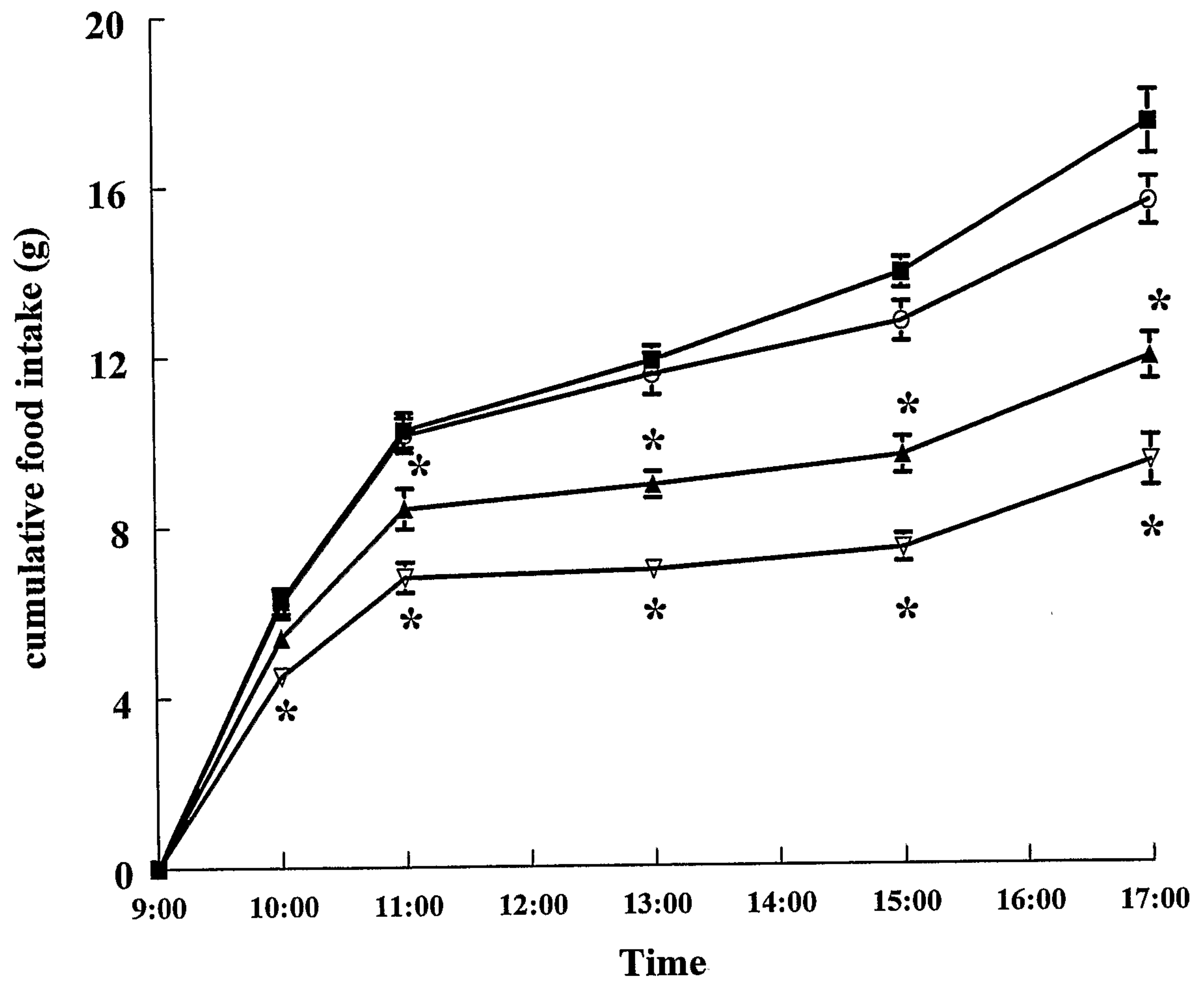


Figure 6a

12/29

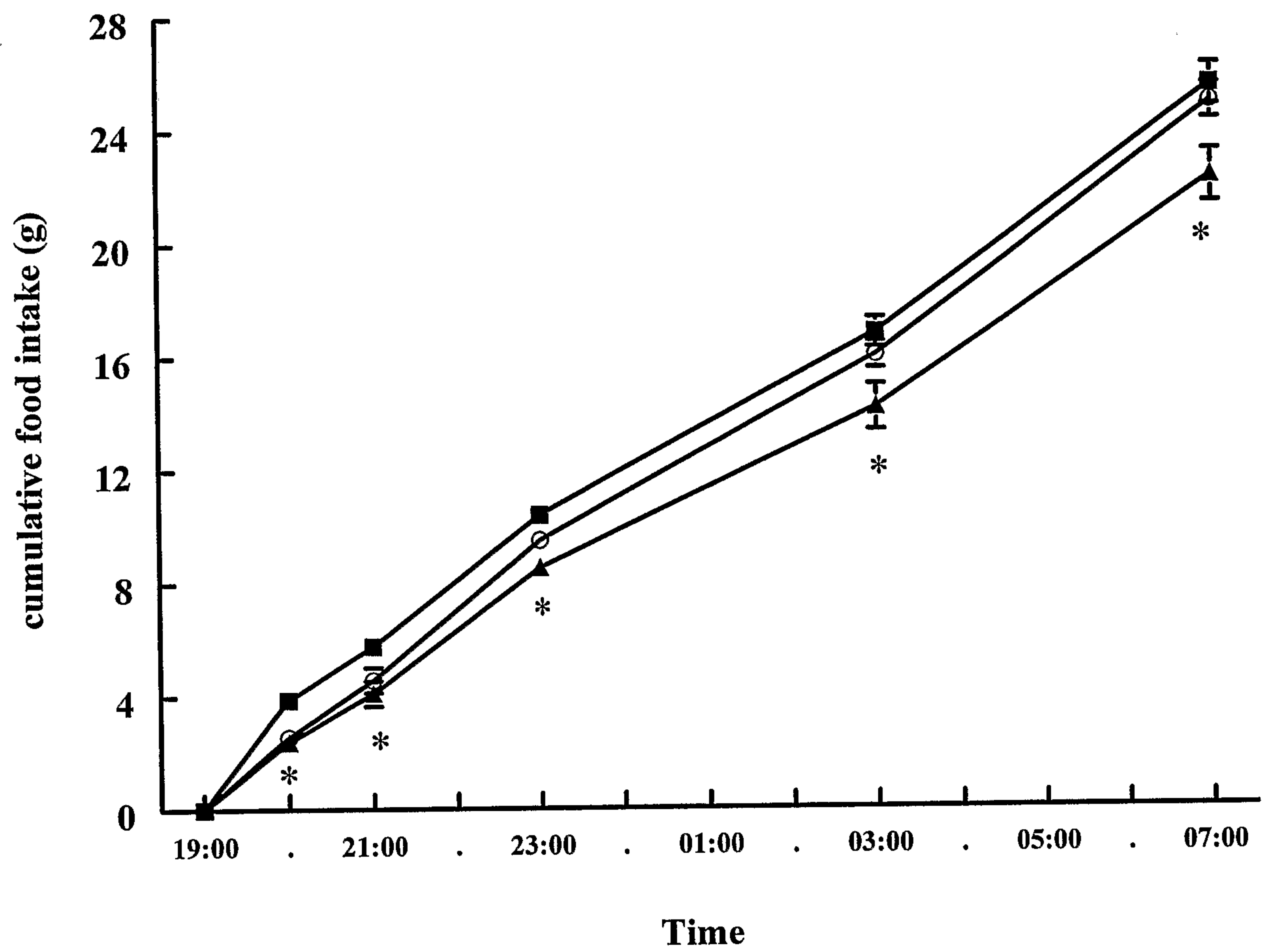


Figure 6b

13/29

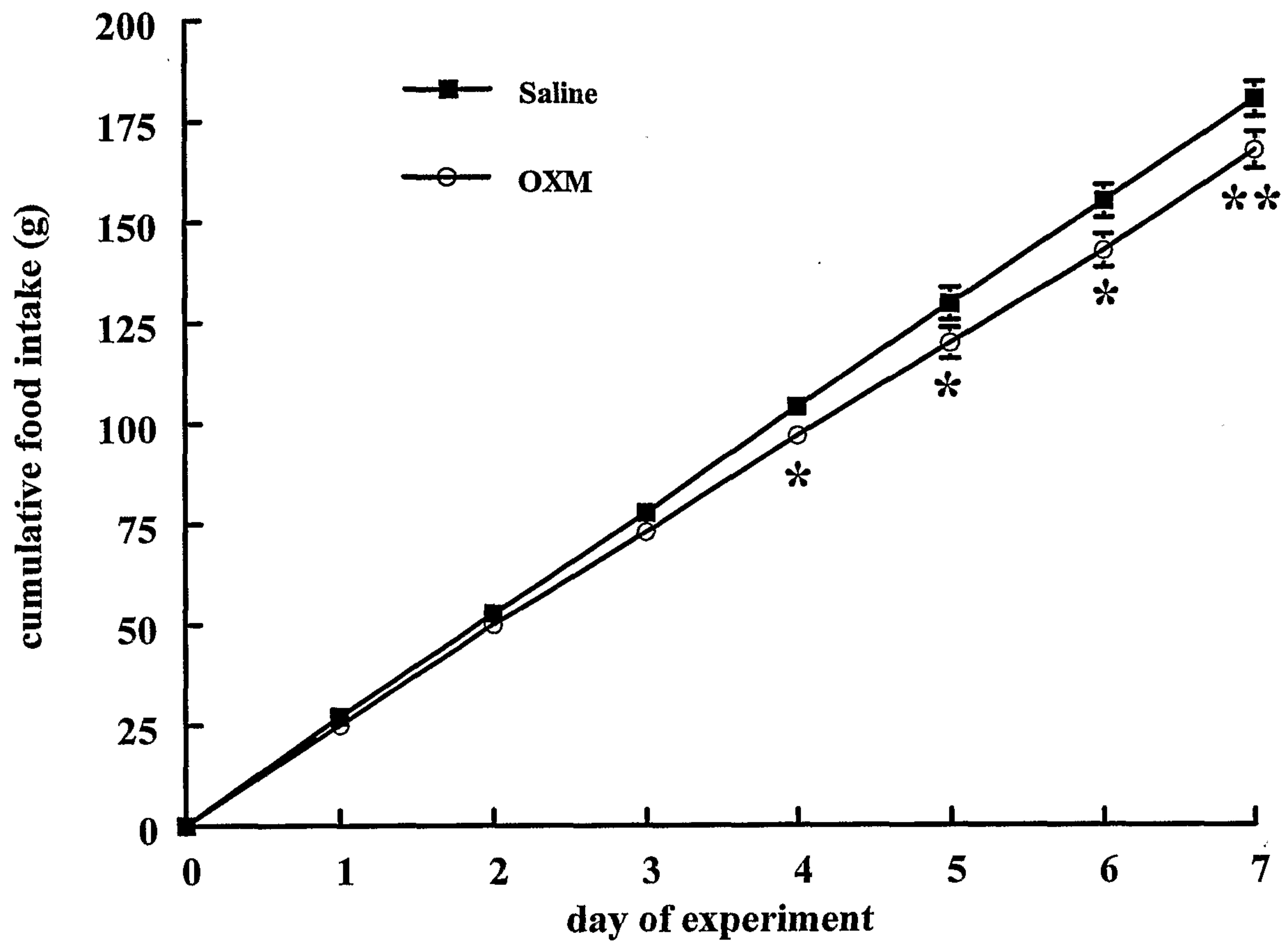


Figure 7a

14/29

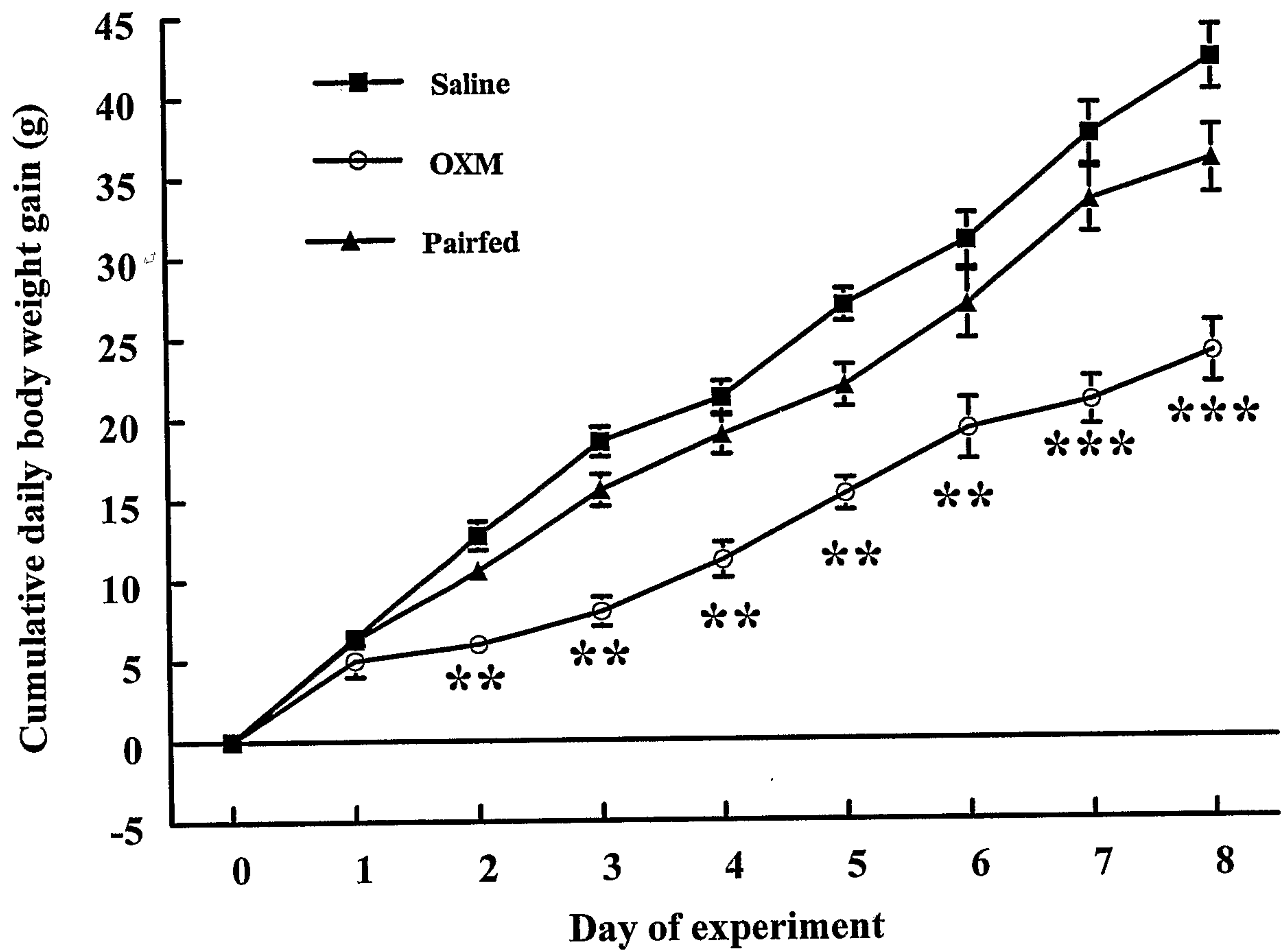
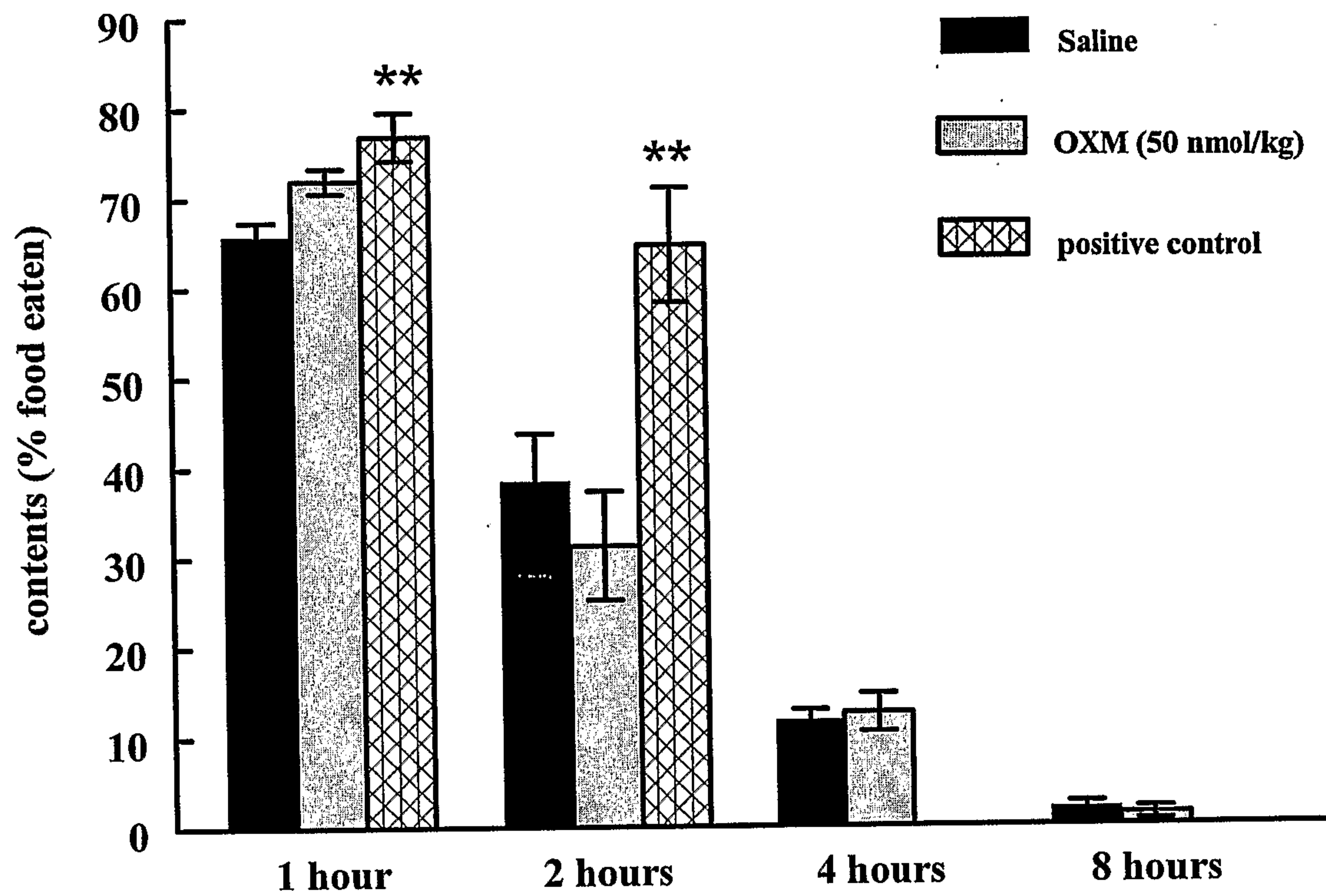


Figure 7b

15/29

**Figure 8****Figure 8**

16/29

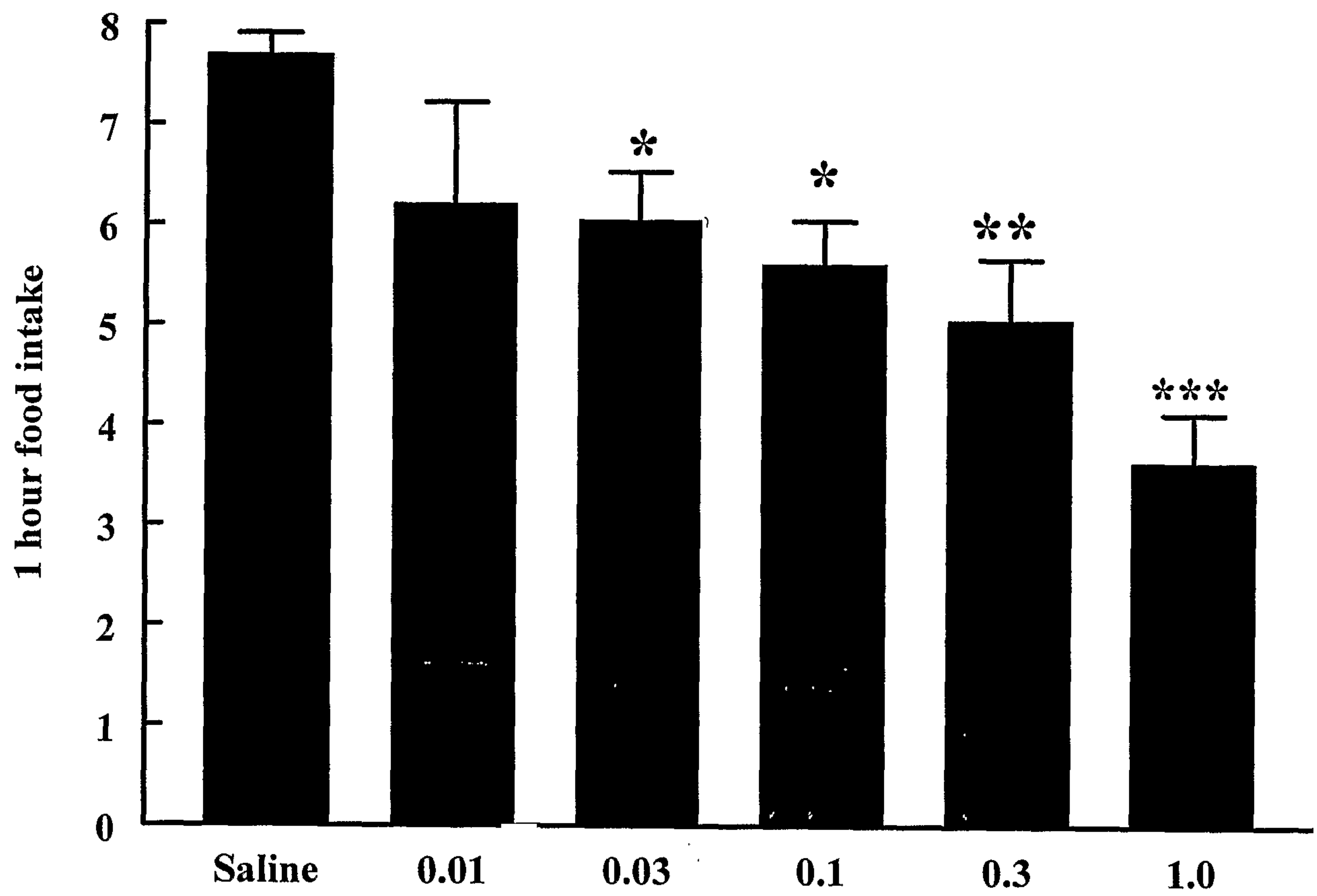


Figure 9

17/29

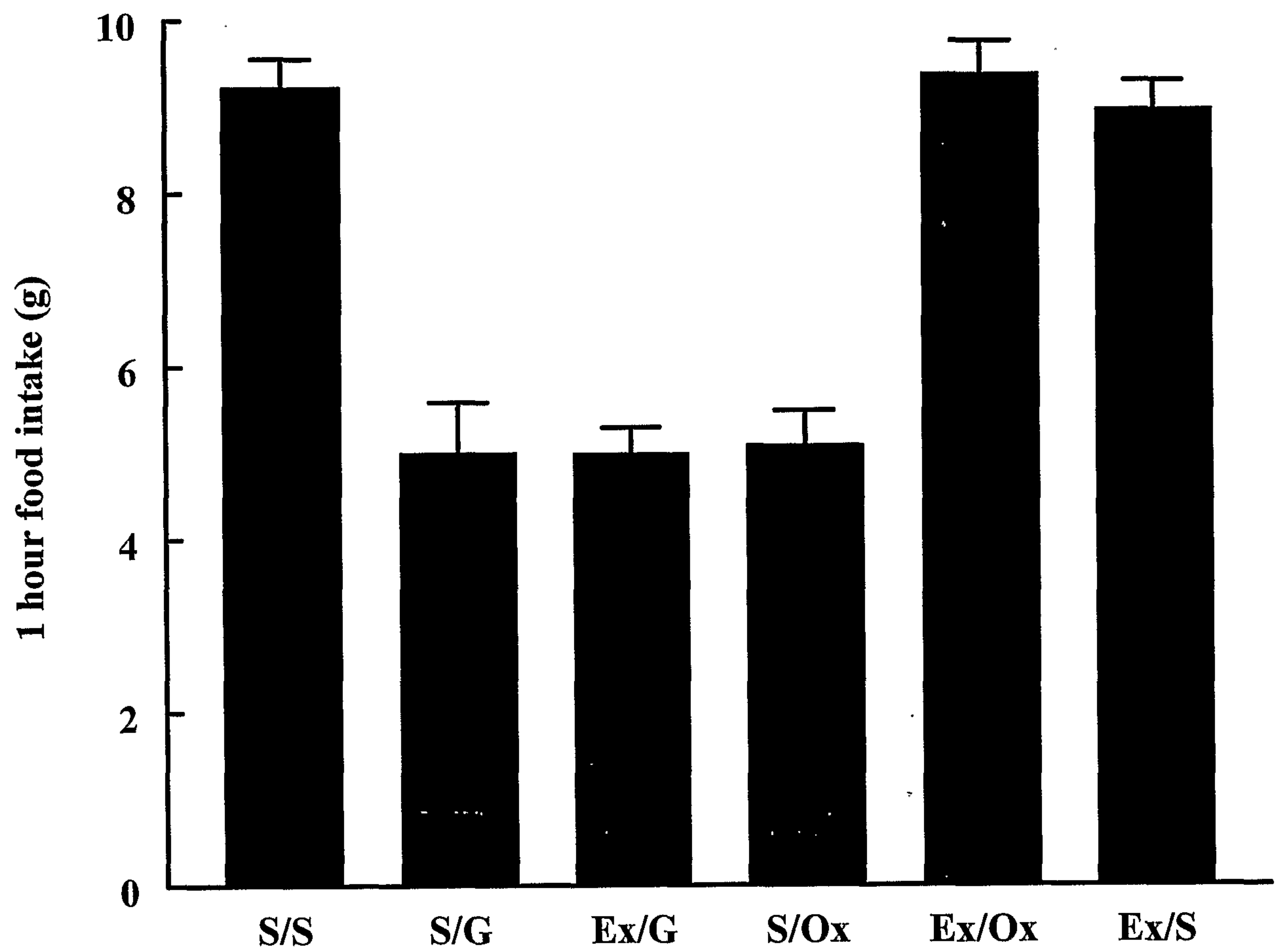
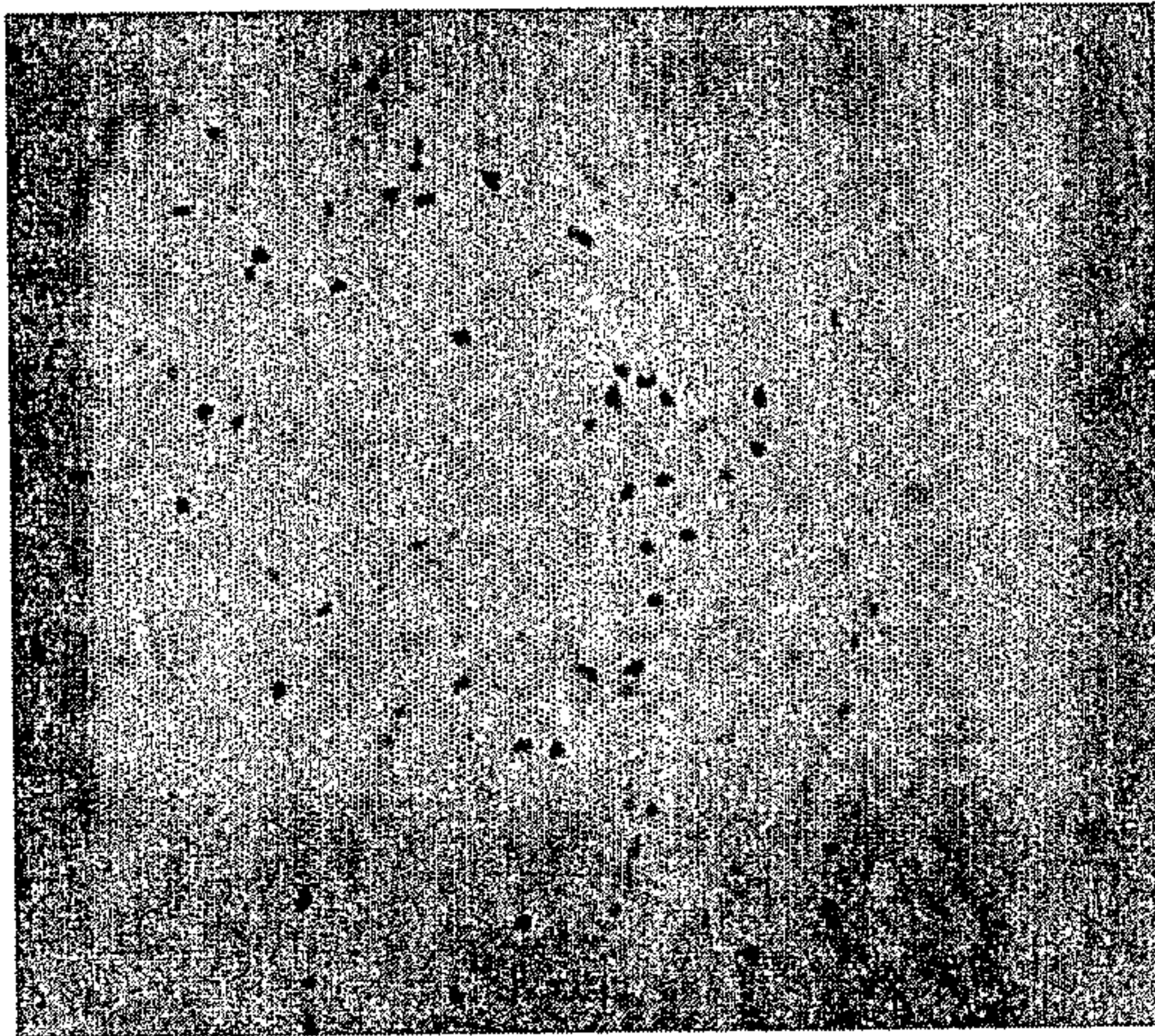
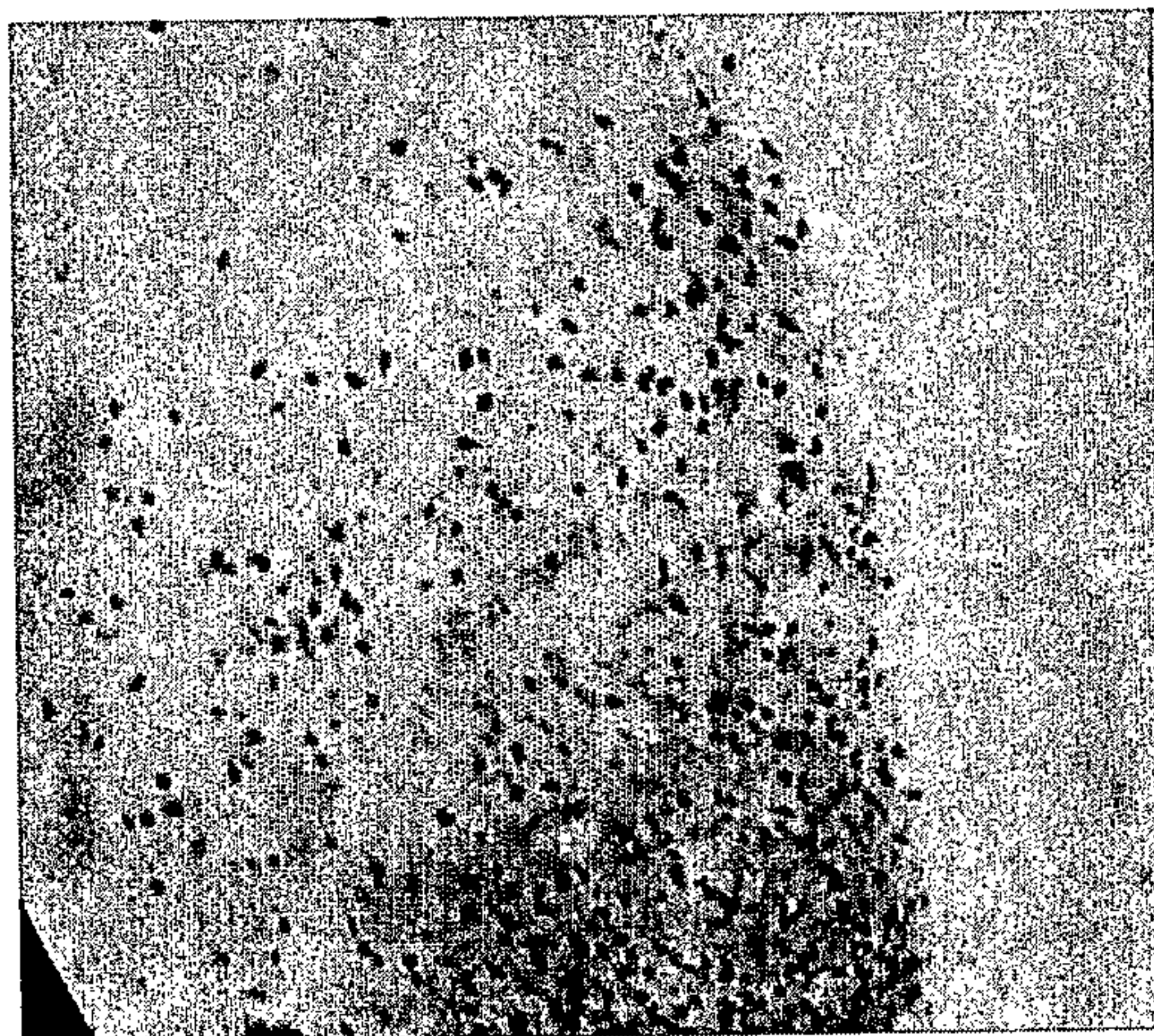


Figure 10

18/29



A



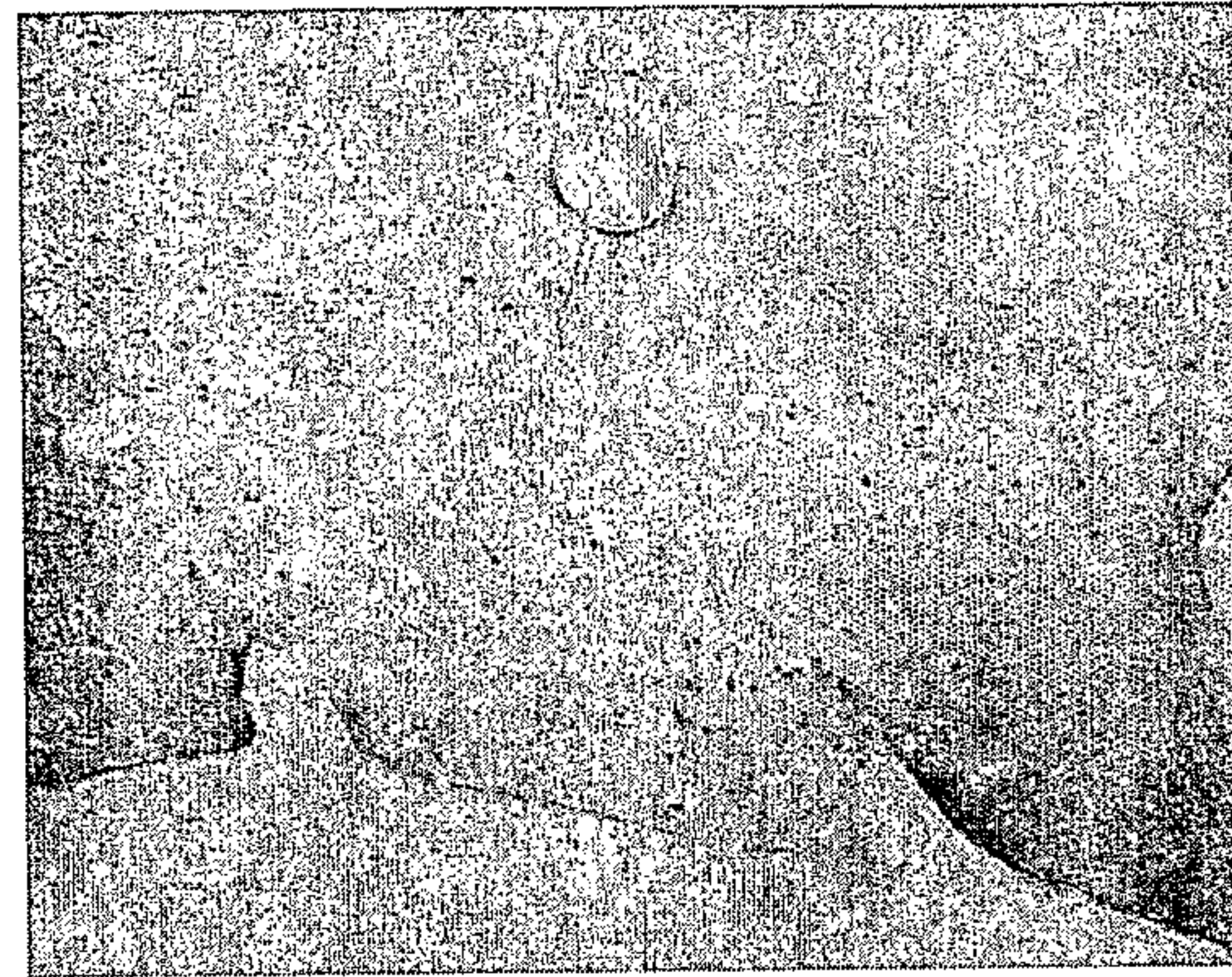
B

**Figure 11a**

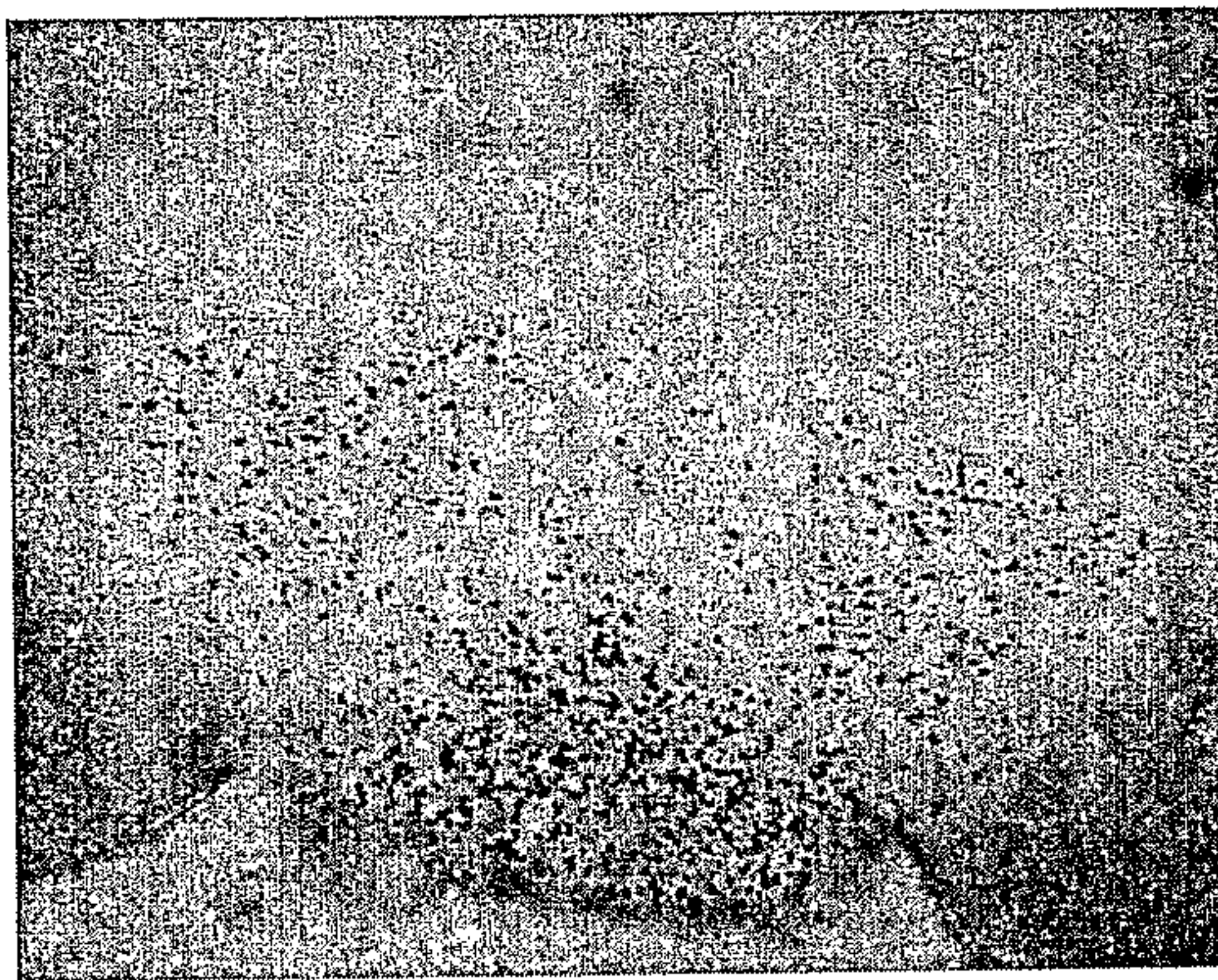
19/29



A)



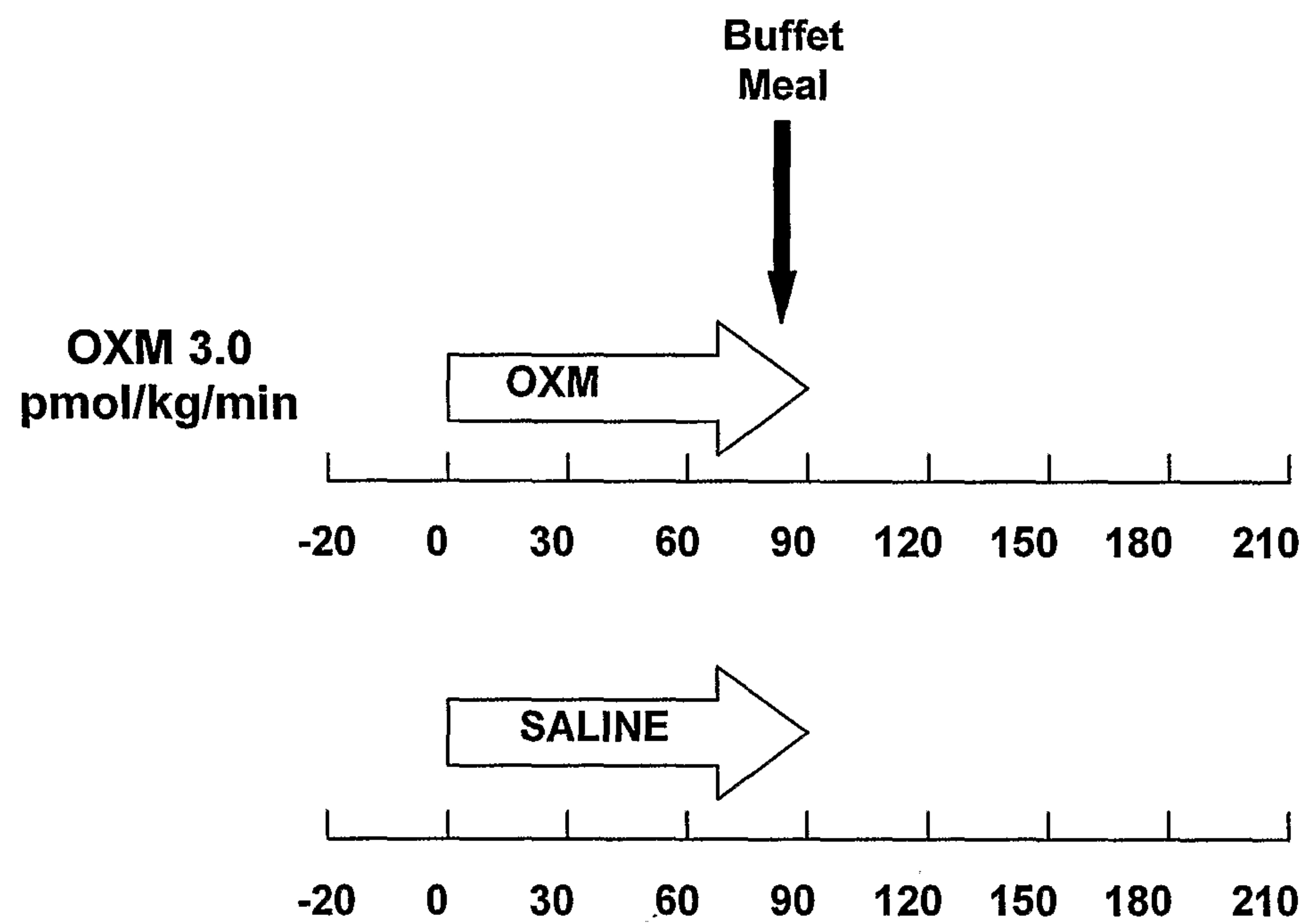
B)



C)

Figure 11b

20/29

**Figure 12**

21/29

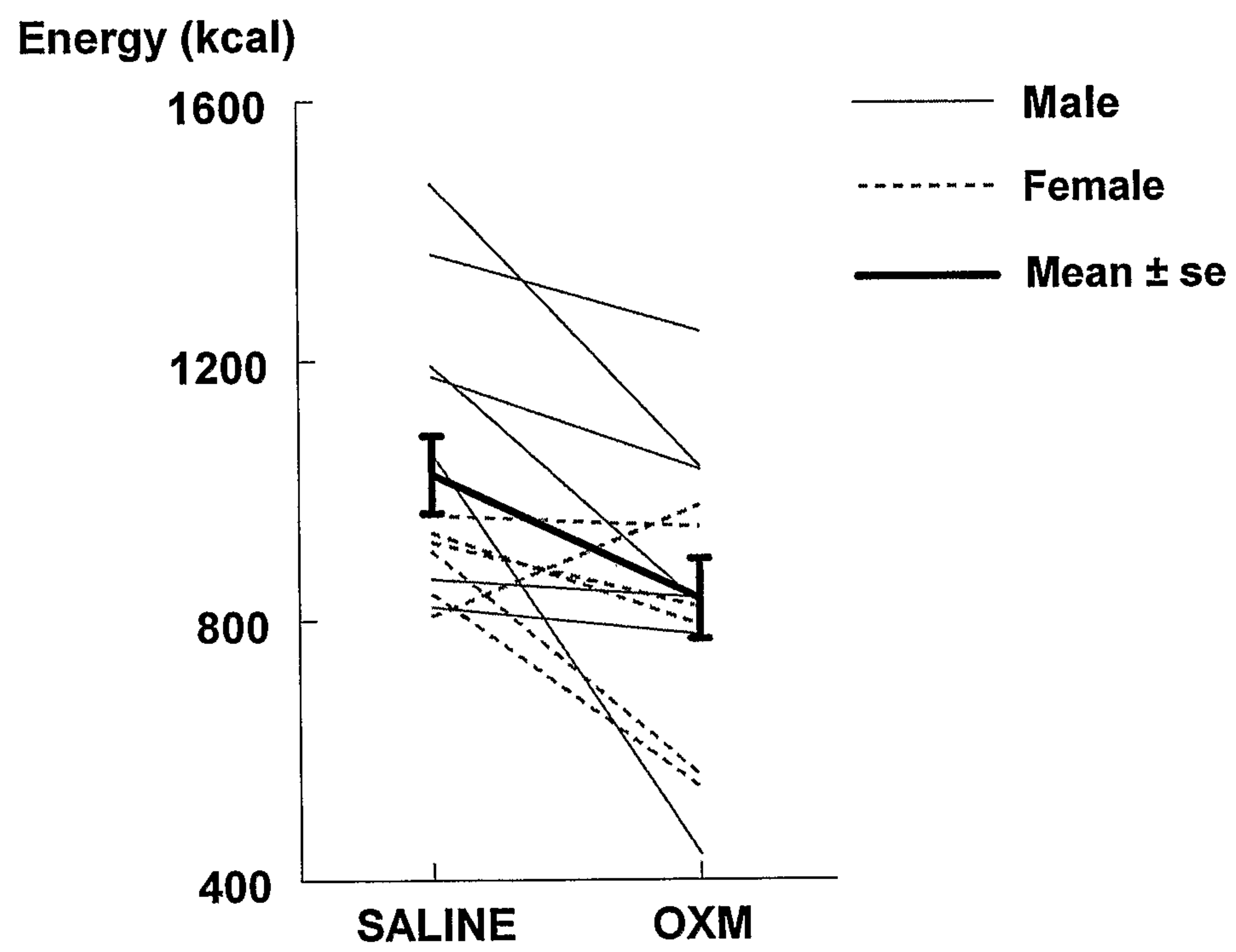


Figure 13

22/29

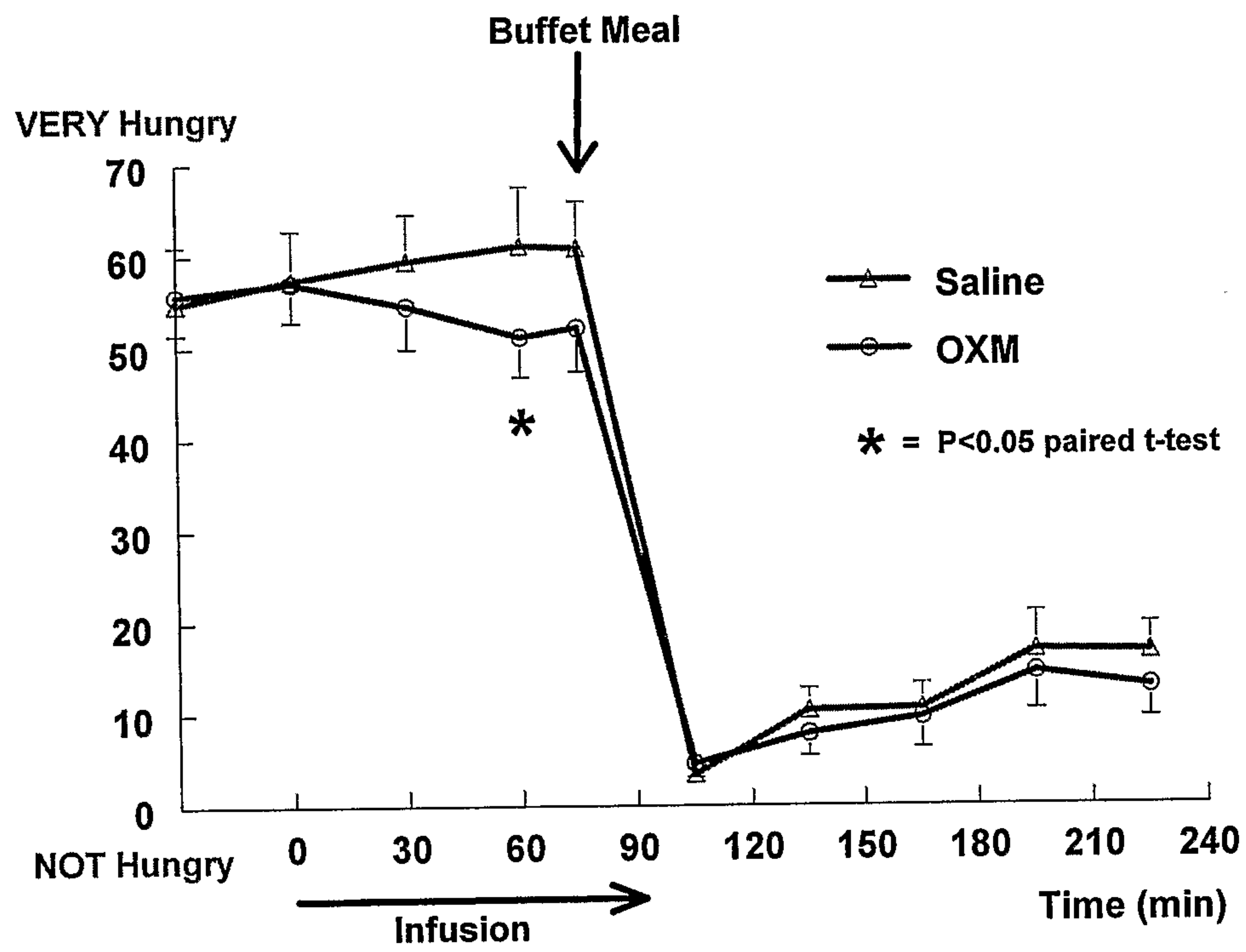


Figure 14

23/29

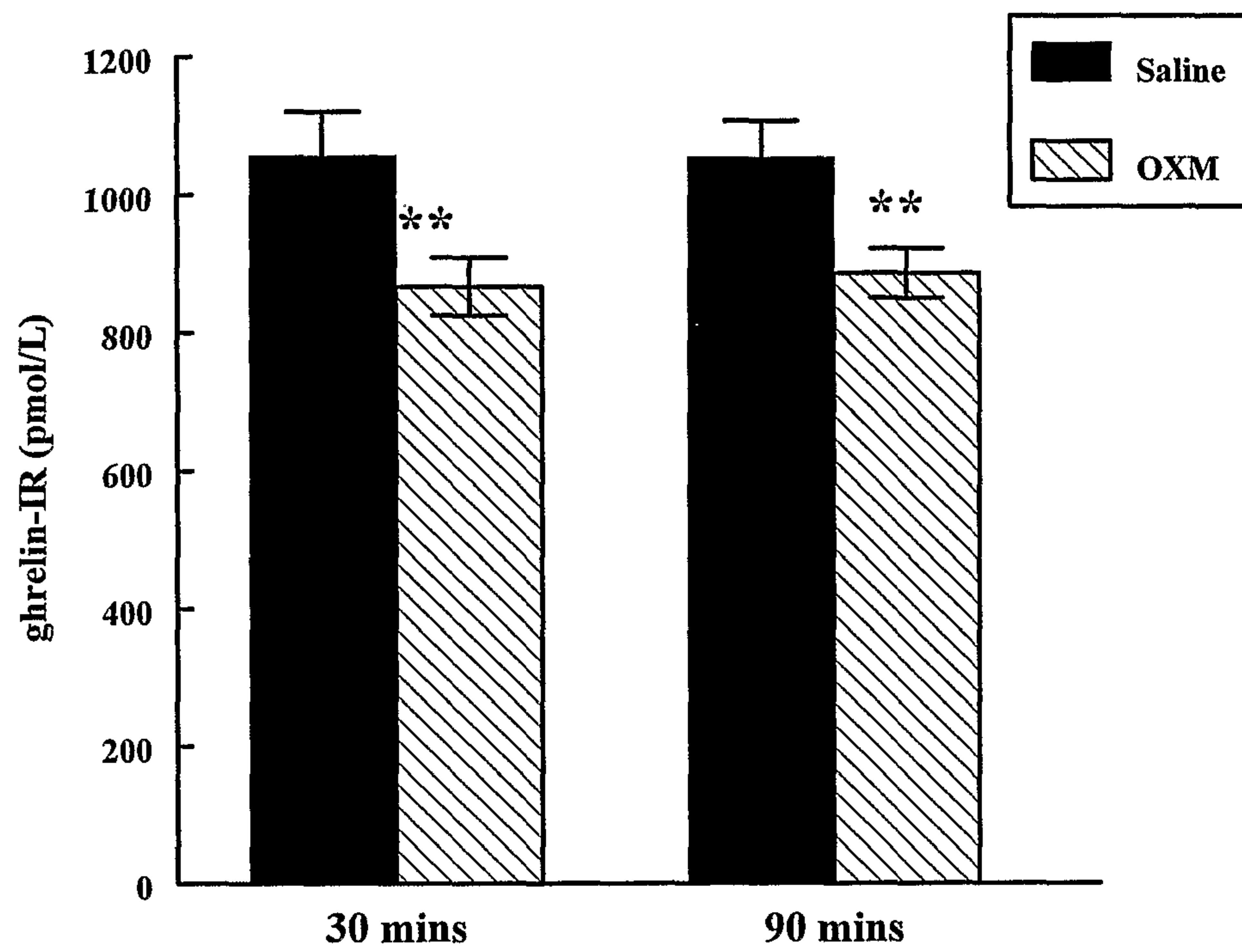


Figure 15

24/29

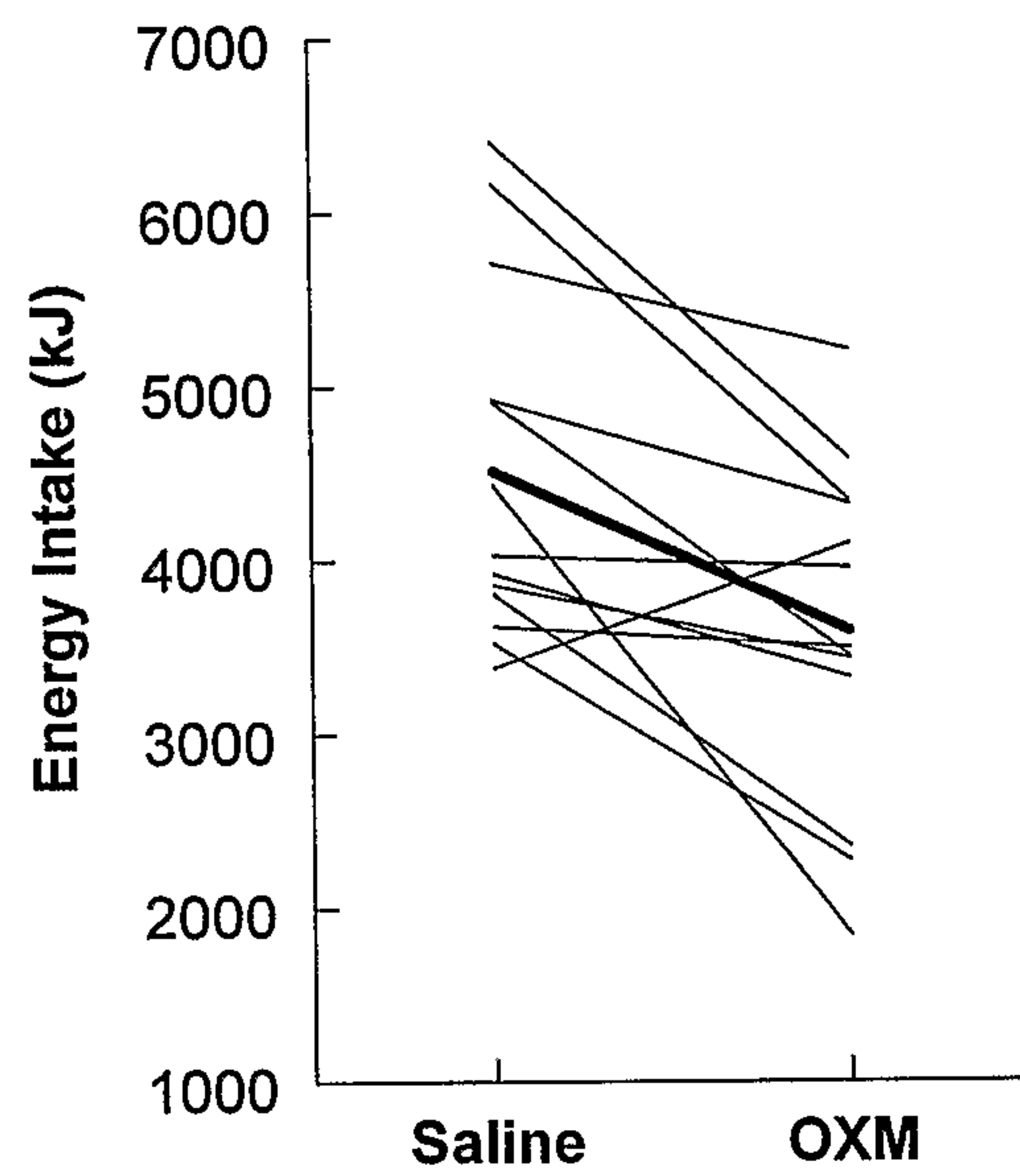


Figure 16

25/29

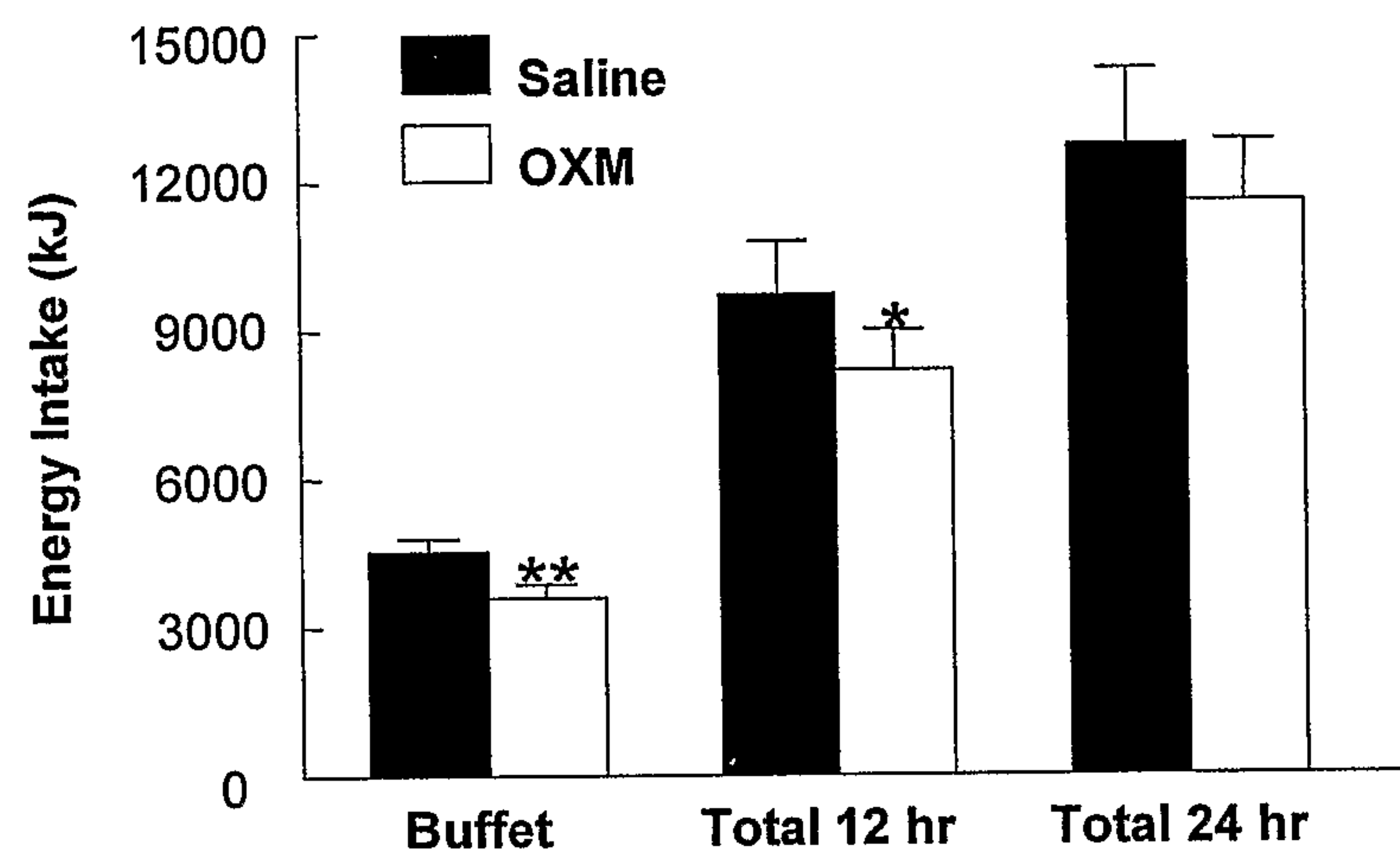


Figure 17

26/29

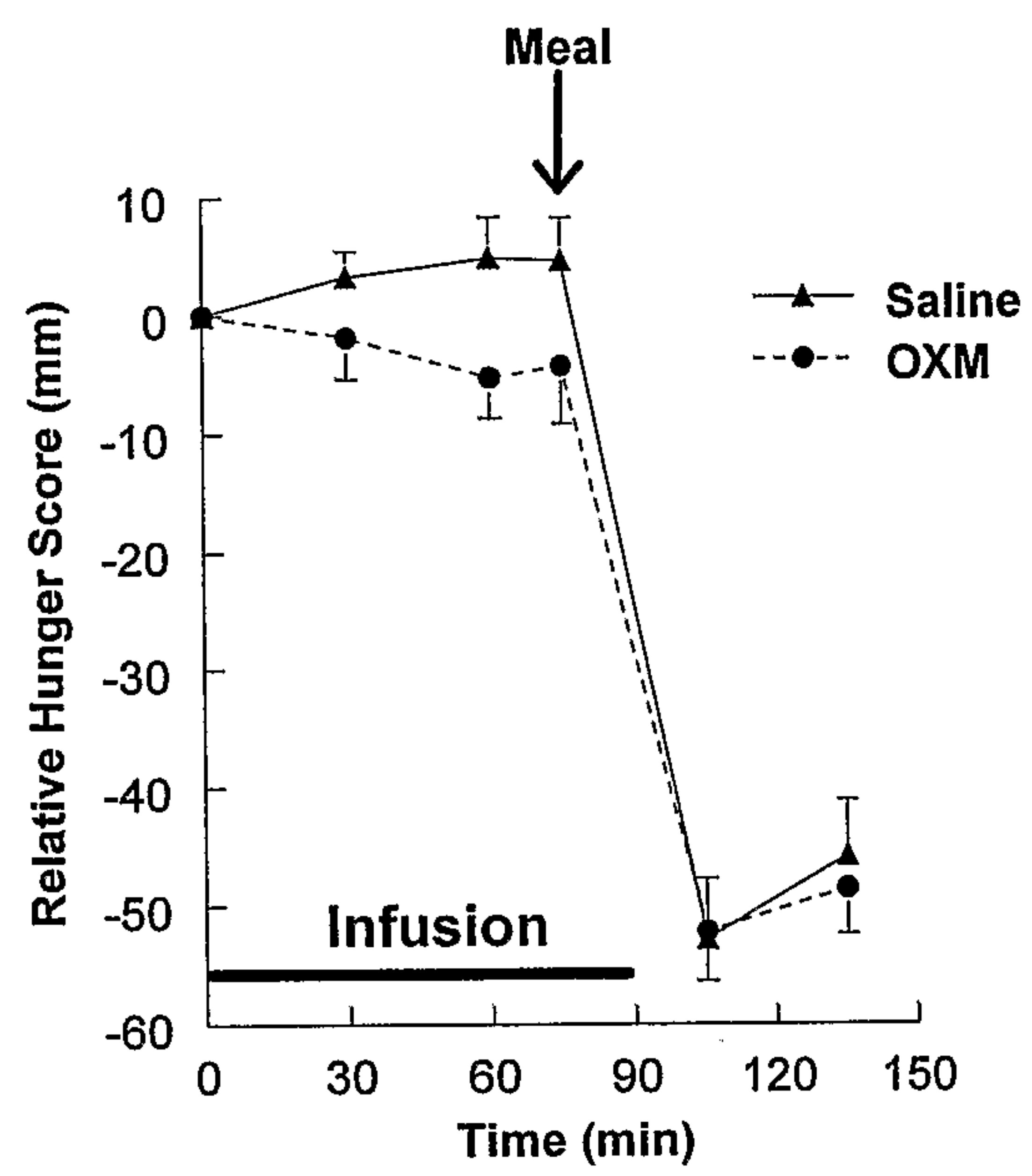


Figure 18

27/29

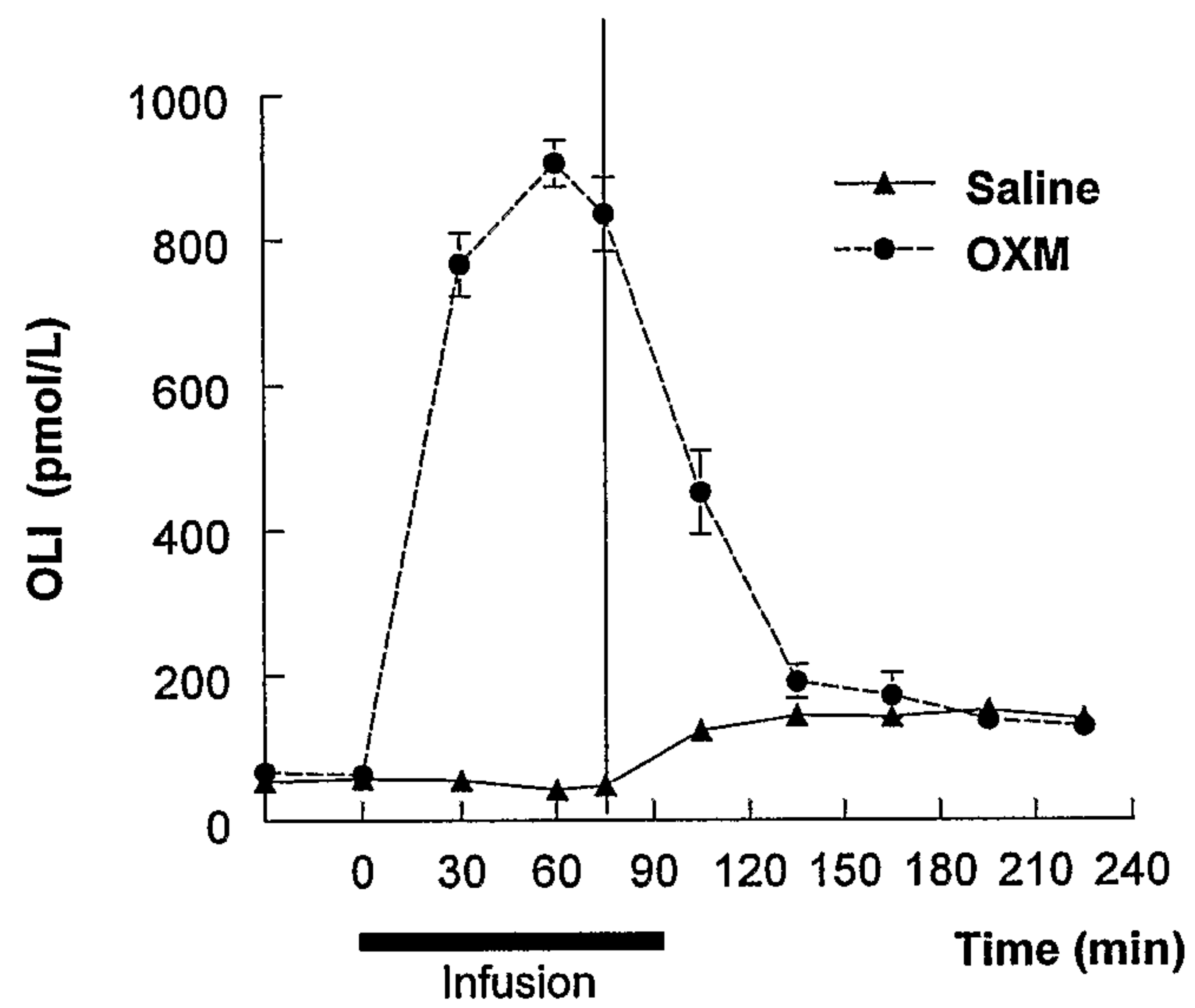


Figure 19

28/29

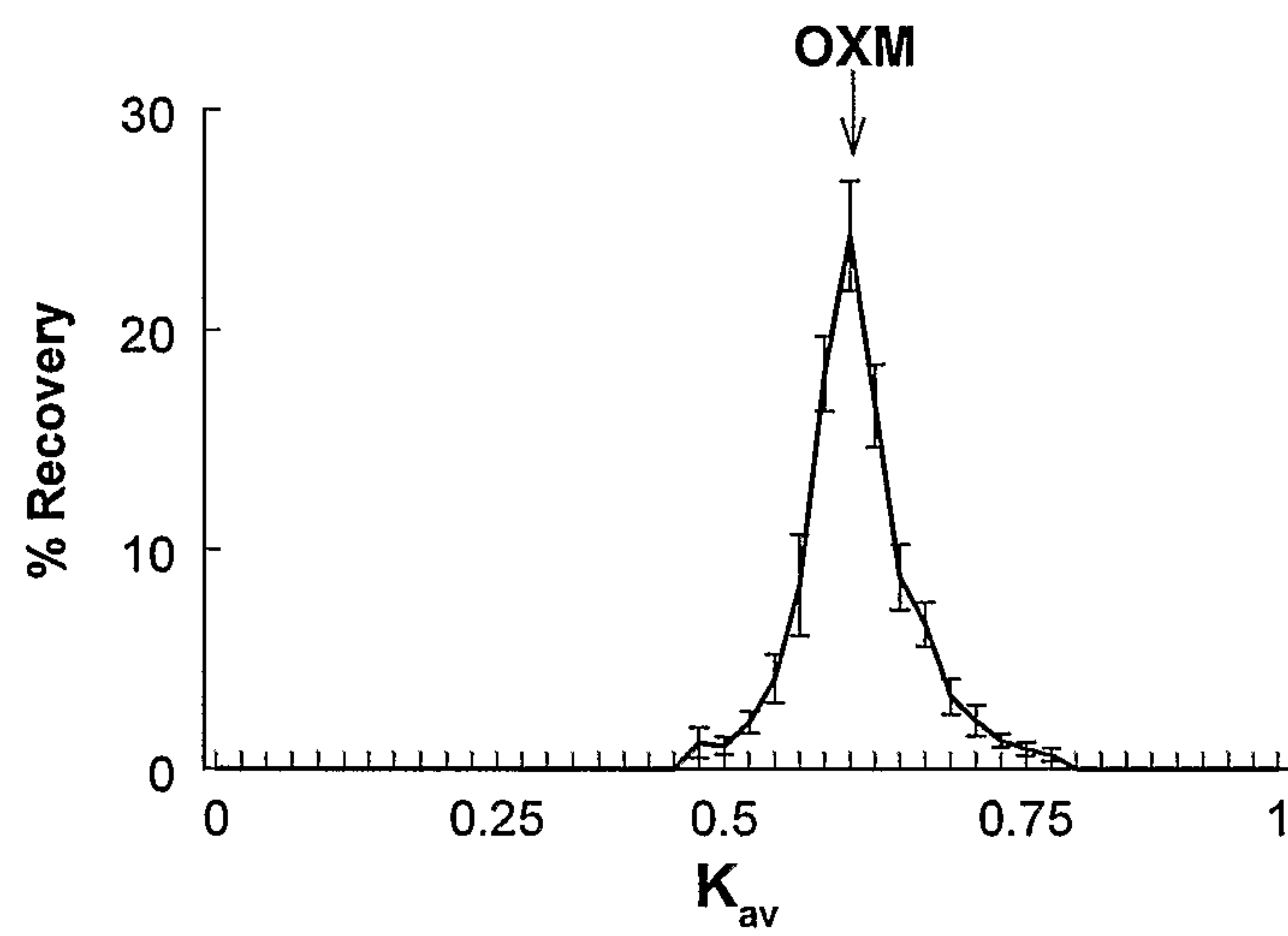


Figure 20

29/29

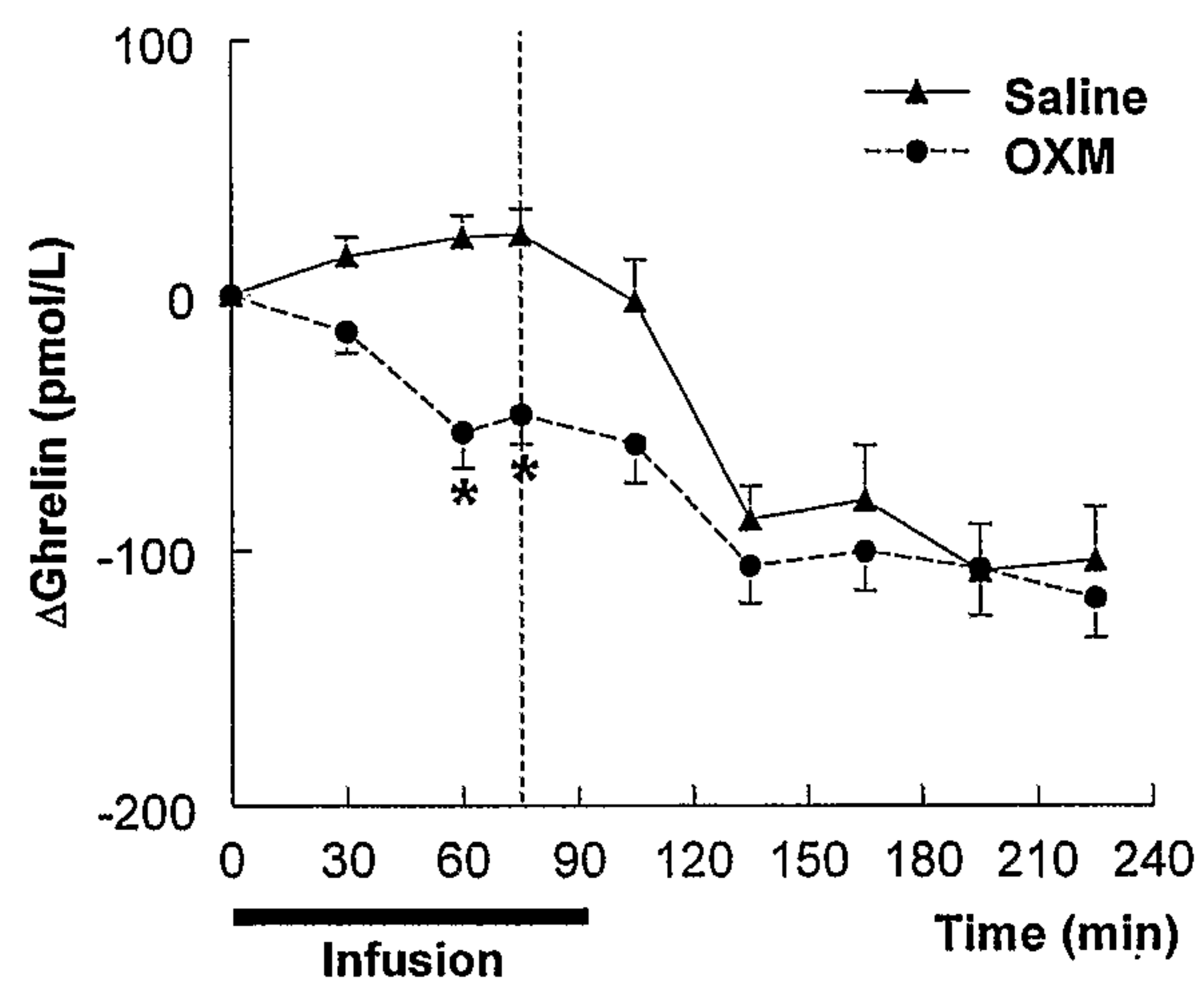


Figure 21