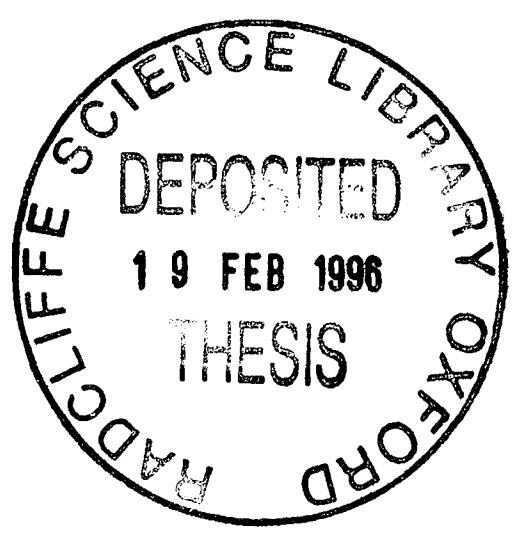
THE ROLE OF SEROTONIN IN THE CONTROL OF MOOD AND APPETITE IN HUMANS

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford.

Hilary Term, 1994



ABSTRACT

The Role of Serotonin in the Control of Mood and Appetite in Humans.

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This thesis addresses the effects of pharmacological manipulations of brain 5-hydroxytryptamine (5-HT, serotonin) and it's precursor, tryptophan, on appetite and mood in humans. Chapter 1 is a presentation of the literature reviewed in order to carry out the studies contained within this thesis. General methods are described in Chapter 2; these include biochemical methods for analysis of plasma tryptophan, and measures and assessment methodologies for analysis of appetite and mood. This chapter also contains a pilot study of the methodology adopted for lowering plasma tryptophan levels.

The first experiment (Chapter 3) examines the effects of calorie controlled dieting on plasma tryptophan, mood and appetite using a longitudinal design. Dieters were compared with a matched control group, and the results demonstrated that whilst dieting does not appear to alter mood or responses to food in a laboratory setting, it does lower levels of plasma tryptophan compared with baseline and with controls. In view of the confounding variables of dieting on mood and appetite, the second experiment (Chapter 4) examined the effects of an acute, laboratory based depletion of plasma tryptophan on these parameters in healthy female volunteers acting as their own controls. Significant depletion of plasma tryptophan was not associated with alterations in mood or appetite.

The third experiment (Chapter 5) addresses the issue of predisposing factors in the effects of tryptophan depletion on mood and appetite. This was carried out with a group of women who had recovered from an eating disorder (bulimia nervosa). These subjects were acting as their own controls but were also compared directly with the non-clinical group of subjects from the previous experiment. This experiment demonstrated interesting differences in the eating behaviour of the two groups, and a significant difference in baseline levels of total plasma tryptophan. There were, however, no effects of tryptophan depletion on mood or appetite in the women who had recovered from bulimia nervosa.

In view of the apparent lack of effect of tryptophan depletion on mood or appetite, the remaining two experiments examine the role of specific 5-HT receptor subtypes in the control of appetite. Chapter 6 examines the effect of meta-chlorophenylpiperazine (mCPP), a 5-HT_{2C} receptor agonist on appetite, and Chapter 7 examines the effect of 5-HT_3 receptor blockade on amphetamine anorexia. Whilst the data from these experiments do not support a role for these receptor subtypes in appetite, it is suggested that this is a potentially fruitful area for future research.

The results generated by the above experiments are discussed in Chapter 8 in the light of other research findings. The methodologies adopted for these experiments and the implications of these studies for future research are discussed.

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GLOSSARY OF ABBREVIATIONS

ACTH	Adrenocorticotrophin
BCAA	Branch chain amino acid
BDI	Beck Depression Inventory
BMI	Body Mass Index
CCK	Cholecystokinin
CHO	Carbohydrate
CNS	Central Nervous System
CSF	Cerebrospinal fluid
DRN	Dorsal raphe nucleus
DA	Dopamine
5,7-DHT	5,7-Dihydroxytryptamine
DZ	Dizygotic
EAT	Eating Attitudes Test
EDE-Q3	Eating Disorder Examination Questionnaire, version 3
FOL	Follicular
GABA	Gamma-aminobutyric acid
GH	Growth hormone
5-HIAA	5-Hydroxyindoleacetic acid
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
5-HT	5-Hydroxytryptamine, serotonin
5-HTP	5-Hydroxytryptophan
HPA	Hypothalamic-pituitary-adrenal axis
KJ	Kilo Joules
kcal	Kilo calories
LH	Lateral hypothalamus
LNAA	Large neutral amino acid
LUT	Luteal
MAOI	Monoamine oxidase inhibitor
MCPP	meta-Chlorophenylpiperazine
MHPG	3-methoxy-4-hydroxyphenylglycol
MRN	Median raphe nucleus
MZ	Monozygotic
NA	Noradrenaline
NPY	Neuropeptide Y
OCD	Obsessive-Compulsive disorder
PCPA	p-Chlorophenylalanine
PRL	Prolactin
PRO	Protein
PVN	Paraventricular nucleus
SEM	Standard error of the mean
SD	Standard deviation
TCA	Tricyclic antidepressant
TFMPP	1-[3-(trifluoromethyl)phenyl]piperazine
TRP	Tryptophan
VMH	Ventromedial hypothalamus

CHAPTER 1 INTRODUCTION

1.1 5-HT, BIOCHEMISTRY, AND BEHAVIOUR

1.1.1 THE ROLE OF 5-HT IN HUMAN BEHAVIOUR

There has been much research examining the role of monoamine neurotransmitters (noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT, serotonin)) in human behaviour. This work has often stemmed from research carried out in clinical populations, but increasingly, psychopharmacological techniques have enabled an exploration of the relationship between neurotransmitter function and normal behaviour. Much of this work has centred on the role of brain 5-HT, and a wide range of behaviours have been implicated. For example, manipulation of brain 5-HT function produces marked changes in sleep (Sharpley and Cowen, 1993), appetite (Blundell et al, 1987; Goodall et al 1991a), aggression (Brown et al, 1982) and sexual behaviour (Gorzalka et al, 1990). 5-HT pathways are widely distributed throughout the brain, especially in the forebrain region (Dahlstrom and Fuxe, 1964), and one reason why 5-HT function modulates so many behaviours is this widespread distribution.

It is clear that the relationship between neurotransmitters and behaviour is highly complex, and it is not possible to consider a particular neurotransmitter in isolation with regard to a particular behaviour. However, some general theories have been put forward which attempt to explain how 5-HT may influence such a diverse range of behaviours. It has been argued, for example, that 5-HT has an inhibitory role in neural information processing (Spoont, 1992). By this proposal, lowered levels of 5-HT activity are associated with a lack of

inhibition of behaviour, thus resulting in increased aggression, startle response, sexual behaviour, and food intake (Spoont, 1992). Conversely, an increase in activity is associated with hyperrigidity in behaviour (Spoont, 1992). Another formulation argues that 5-HT systems act to reduce the impact of impending aversive events (Deakin, 1991). This model conceptualises a two system mechanism where projections from the dorsal raphe nucleus mediate fear and anxiety in response to an aversive event. The median raphe nucleus system is associated with adapting to aversive events once they have occurred. Deakin (1991) has proposed this model to explain certain features of clinical anxiety and depression. A further theory put forward by Jacobs and Fornal (1993) proposes that, under normal conditions, the primary function of 5-HT neurones is to facilitate motor output. This system further acts in an ancillary fashion to concurrently inhibit sensory information processing, and to coordinate autonomic and neuroendocrine function in relation to changing motor output.

These models serve to illustrate that 5-HT pathways in widespread distribution interact with the modular structure and distributed neuronal circuit of the brain. In this way, 5-HT is able to influence a wide range of behaviours. However, the 5-HT system itself, is not homogenous, and shows evidence of morphological and functional specialisation (see section 1.2). The effect of 5-HT is also expressed through multiple receptor sub-types, thus providing an additional level of complexity in which 5-HT can modulate various behaviours. Thus, as far as specific behaviours are concerned, it is important to understand what is known about the local 5-HT innervation of the brain region that underpins the behaviour, and also the functional role of different receptor subtypes in modulating the behaviour in question.

1.1.2 TRYPTOPHAN, TRANSPORTATION, AND 5-HT SYNTHESIS

Tryptophan is one of the essential amino acids, and is therefore obtained from dietary sources. Plasma tryptophan is either incorporated into protein tissues, or is metabolised via the 5-hydroxyindole pathway leading to the neurotransmitter amine 5-HT, or it may be metabolised via the oxidative pathway leading to the synthesis of nicotinamide nucleotides (Bender, 1982).

All of the large neutral amino acids (LNAA's) including tryptophan are transported to the brain across the blood-brain barrier (ie, the branch chain amino acids (isoleucine, leucine and valine), and the two aromatic amino acids (phenylanalanine and tyrosine) (Wurtman, 1978). Tryptophan, therefore, is in competition with the other large neutral amino acids for the carrier to the brain where amine synthesis occurs (Oldendorf et al, 1976). Thus, it is the ratio of tryptophan to other LNAA's which determines the amount of tryptophan being transported. This regulatory system is therefore important in controlling levels of brain tryptophan.

Tryptophan binds to serum albumin in the L-isomer form (Bender, 1982). Under normal physiological conditions only about 10% of plasma tryptophan remains unbound (free tryptophan). However, tryptophan is only loosely bound to albumin, and is rapidly stripped off its binding site when passing through the capillaries around the brain (Yuwiler et al, 1977). Non-esterified fatty acids compete with tryptophan for albumin binding, and will therefore displace tryptophan from these sites, thus increasing the availability of free tryptophan (Curzon et al, 1973).

It has been argued, firstly, that it is the ratio of total plasma tryptophan to competing amino acids which is important in determining the rate at which tryptophan will enter the brain (Wurtman, 1978) and secondly, that the proportion of plasma free tryptophan is more important (Bloxam and Curzon, 1978). Fernstrom et al (1976) have compared brain tryptophan in the rat with i) plasma free tryptophan, ii) plasma total tryptophan and iii) the ratio of both free and total to other LNAA's. They report that the best correlations of brain function were with measures of total tryptophan rather than with free tryptophan.

In direct conflict with these findings are those which support the position adopted by Curzon (1985). It has been shown that in refeeding experiments in the rat, that the ratio of total plasma tryptophan to LNAA's remains constant (Curzon, 1988). There was an overall decrease in brain tryptophan, in plasma free tryptophan and in the ratio of this to LNAA's. In contrast there was no change in the ratio of plasma total tryptophan to LNAA's and more importantly, an **increase** in plasma total tryptophan (Sarna et al, 1984). Thus, it has been argued that measures of plasma total tryptophan are not good indices of brain tryptophan.

This pattern of findings has also been demonstrated in humans using CSF tryptophan as an index of brain tryptophan (Perez-Cruet et al, 1974). This issue remains largely unresolved and it is not clear under which particular physiological conditions these different variables become significant. It is important to note that a wide range of different methodologies have been adopted, and the issue of food-deprived versus free feeding animals is responsible for a proportion of the differences in these experimental findings. These findings do, however, reflect the enormous complexity of this system, and the potential interaction between nutrient status and neurotransmitter regulation.

5-HT synthesis

Monoamine neurotransmitter synthesis is controlled by the hydroxylase enzymes. For 5-HT, the first step in the synthesis process is the hydroxylation of tryptophan to 5-hydroxytryptophan (5-HTP). This is catalyzed by the enzyme tryptophan hydroxylase which is confined to the 5-HT neurons (Lovenberg et al, 1968). Under normal physiological conditions, tryptophan hydroxylase is only half saturated with tryptophan and the conversion of tryptophan to 5-HTP is the rate limiting step in the synthesis process. Thus, levels of brain tryptophan affect the degree of enzyme saturation and hence, the rates of tryptophan hydroxylation and 5-HT formation (Grahame-Smith, 1971; Ashcroft et al, 1965; Fernstrom et al, 1971). 5-HTP is then converted to 5-hydroxytryptamine (5-HT) by aromatic-L-amino acid decarboxylase (Lovenberg et al, 1968). 5-HT is taken up and stored in granules at the presynaptic nerve ending.

A two step reaction metabolises 5-HT to 5-hydroxyindoleacetic acid (5HIAA); oxidation of 5-HT by monoamine oxidase (MAO) produces 5-hydroxyindoleacetaldehyde. This is then either dehydrogenated by aldehyde dehydrogenase to 5-HIAA or reduced by aldehyde reductase to 5-hydroxytryptophol (5-HTOH). There are other minor metabolic pathways which include conjugation to a sulphate derivative and action by N-methyl transferase to methylated compounds such as bufogenin.

1.1.3 5-HT NEUROANATOMY AND CLASSIFICATION

5-HT Pathways

The 5-HT neuron is phylogenetically very old and has been identified in the nervous

systems of very simple to highly complex organisms (Azmitia, 1987). 5-HT cell bodies are situated mainly in the raphe nuclei in the brainstem, with a basic pattern common to all mammals studied (Takeuchi, 1988). Dahlstrom and Fuxe (1964) classified indoleamine containing neurones into nine groups in the rat brain (designated B1-B9) (Fig 1.1). The projections of the raphe nuclei are organised into two distinct systems; studies have demonstrated that the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) have differing patterns of innervation of forebrain regions with distinct morphology of their axon terminals (Kohler, 1982; Kohler and Steinbusch 1982; O'Hearn and Molliver, 1984). Ascending projections from the MRN are found preferentially in the hippocampus and septum, and are distinguished by large varicosities giving a beaded appearance (Kofosky and Molliver, 1987). In contrast, projections from the DRN project mainly into the cortex and striatum, and are characterised by fine axons which are particularly sensitive to the neurotoxic effects of amphetamine derivatives (O'Hearn et al, 1988). The projections of the DRN to the cortex are particularly associated with 5-HT_2 receptors (Molliver, 1987). 5-HT neurones in the DRN stimulate prolactin secretion (Van de Kar and Bethea, 1982), and the nerve terminals involved in this response are located in the hypothalamus (Van de Kar, 1985). The hypothalamus, which is an important structure in the regulation of feeding behaviour, is innervated by both systems.

These findings suggest that 5-HT innervation of the cortex and limbic system does not function in an homogeneous way, but consists of distinct neuronal projections with high degrees of anatomical and morphological specialisation. Such organisation may well have major implications for the understanding of different types of behaviour, and the opportunity exists to examine this with selective pharmacological agents (Cowen, 1991).

Fig 1.1



The distribution of the main serotonin-containing pathways in the rat central nervous system. From Cooper et al, 1986.

5-HT Receptors

The last 15 years have witnessed a large increase in the understanding of neurotransmitter receptors and their subtypes. It is clear that 5-HT receptors exist in multiple biochemical subtypes which mediate different behavioural effects (Goodwin and Green, 1985; Peroutka et al, 1981). Fig 1.2 depicts a simple 5-HT neurone.

Gaddum and Picarelli (1957) first reported that 5-HT interacted with at least two distinct receptors in the guinea-pig. Ligand binding studies led to further classification of the two CNS 5-HT receptors. 5-HT_1 receptors were characterised by nanomolar affinity for 5-HT, and 5-HT_2 receptors by micromolar affinity for 5-HT but nanomolar affinity for spiperone (Peroutka and Snyder, 1979). Subsequent classification by Bradley et al (1986) included a 5-HT_3 family, where each of these receptor families are distinguished by their second messenger system, ie that which mediates the intracellular response to receptor activation (Cowen, 1991). However, recently the classification has been re-appraised by the Serotonin Club Receptor Nomenclature Committee who have reclassified the subtypes under a more comprehensive system (Humphrey et al, 1993). This includes the recently identified 5-HT_4 receptor which is positively linked to adenyl cyclase (Bockaert et al, 1990).

Fig 1.2



General model of brain serotonin neurone; depiction of many common elements. Different types of 5-HT neurone have different morphology, co-transmitters (neuropeptides and monoamines), and post-synaptic receptors on different target neurone cell bodies and terminals. (TP = L-tryptophan; Co-T = co-transmitters and modulators ie neuropeptides and monoamines other than 5-HT; TH = tryptophan hydroxylase; AADC = L-amino acid decarboxylase; MAO = monoamine oxidase; CAMP = cyclic adenosine monophosphate; Pi = phosphatidylinositol). (From Murphy, 1991)

This thesis adopts the new classification system which is set out in table 1.1 alongside the traditional system.



5-HT_1 receptor family

The 5-HT_1 receptor has been subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F} (Humphrey et al, 1993; Heuring and Peroutka, 1987). Recent reclassification has moved the 5-HT_{1C} receptor to the 5-HT_2 family and renamed this receptor as 5-HT_{2C}. The 5-HT_1 receptor family share nanomolar affinity for 5-HT and 5-carbamidiotryptamine and micromolar affinity for 5-HT antagonists such as mesulergine. Recently, specific radioligands have been developed for the 5-HT_{1A} receptor, the prototype being 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) (Gozlan et al, 1983). The pyrimidinyl piperazines

(buspirone, gepirone, and ipsapirone) are also selective ligands at the 5-HT_{1A} receptor (Traber and Glaser, 1987).

5-HT_{1A} receptors are regarded phylogenetically as being the oldest member of this family, and have a heterogeneous distribution with high densities in the hippocampus and raphe nuclei (Pazos et al, 1988). 5-HT_{1B} receptors are species specific and are present with high densities in the basal ganglia in the rodent (Pazos et al, 1988). The human homologue for this is the 5-$HT_{1D\beta}$, although this differs from the rat homologue by having 5-HT_{1D}-like operational characteristics (eg ß-adrenoceptor antagonists have low affinity for these receptors) (Humphrey et al, 1993). 5-HT_{1D} receptors are present in the striatum, substantia nigra, neocortex, and hypothalamus (Pazos et al, 1988).

The 5-HT_1 receptor family are G protein-linked and activation leads to the inhibition of adenylate cyclase (Peroutka, 1988). 5-HT_{1A} receptors are found on the cell bodies of the neurone in high concentrations in the raphe nuclei and at post-synaptic locations in the hippocampus, amygdala, and certain hypothalamic nuclei (Pazos et al, 1988). The 5-HT_{1A} autoreceptors on the cell bodies have an inhibitory effect of 5-HT neurones, whereas post-synaptic 5-HT_{1A} receptors mediate the effects of 5-HT released from nerve terminals (Cowen, 1991). 5-HT_{1B} and 5-HT_{1D} receptors appear to act as presynaptic terminal autoreceptors (Hoyer and Middlemiss, 1989).

5-HT_2 receptor family

This family consists of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (previously classified as 5-HT_{1C}). These receptors share a common second messenger system linked to G proteins which

involves the hydrolysis of phosphoinositides and the mobilisation of intracellular calcium (Hoyer, 1988a; Hartig, 1989). 5-HT_{2C} receptors are found in choroid plexus, basal ganglia, cerebral cortex , hippocampus and hypothalamus. 5-HT_{2A} receptors are found in high concentrations in the cerebral cortex, certain regions of the amygdala, cingulate and hypothalamus (Pazos, 1988). Receptors in this family appear to be located post-synaptically.

5-HT_3 receptor family

5-HT_3 receptors belong to the ligand-gated ion channel receptor superfamily (Humphrey et al, 1993). It is suggested that many subunits exist, although none of these have been cloned (Humphrey et al, 1993). Peripheral 5-HT_3 receptor sites have already been well characterised, but the CNS binding sites have only relatively recently been demonstrated (Kilpatrick et al, 1987). The highest densities are in the cortical areas and the dorsal vagal complex. 5-HT_3 receptors differ from 5-HT_1 and 5-HT_2 receptors in that they are directly coupled to an ion channel with no intervening G protein or second messenger system (Peroutka, 1988). The highest CNS concentrations of receptor binding sites is found in the area on the nucleus tractus solitarius in the brainstem (Pratt et al, 1990). There are also lower densities of binding sites in areas of the limbic system including the hippocampus and amygdala, and also the entorrhinal cortex (Kilpatrick et al, 1987).

5-HT_4 receptor family

No structural information on 5-HT_4 receptors has yet been published. This receptor is thought to be G protein-linked (Humphrey et al, 1993), and is positively coupled to

adenylate cyclase (Dumuis et al, 1988). No further information on operational characteristics is yet available.

1.1.4 MEASURES OF BRAIN 5-HT FUNCTION IN THE HUMAN

In animals, brain 5-HT and metabolites such as 5-hydroxyindoleacetic acid (5-HIAA) over specific binding sites can be measured directly. This is carried out using methods of receptor binding, biochemical and electrophysiological techniques. Direct measures of central 5-HT metabolism is not possible in the human brain, and a variety of indirect methodologies for measuring 5-HT function are employed.

Cerebrospinal Fluid (CSF) 5-HIAA

In this methodology, CSF obtained from the lumbar space is measured for concentrations of 5-HIAA. This is, however, an invasive technique, and has been further criticised as not necessarily reflecting events occurring in higher parts of the CNS (Bulat, 1984).

Post mortem 5-HT receptor binding

Receptor binding studies offer an index of receptor numbers in particular brain regions. The imipramine binding site is thought to be located on the 5-HT nerve terminals associated with the 5-HT uptake site or transporter (Paul et al, 1984). Studies of [^{3}H]-imipramine binding may therefore provide an index of 5-HT uptake site numbers and/or function. In addition, studies employing the technique of positron emission tomography (PET) enables the measurement of 5-HT receptors. Specifically PET studies have enabled the

measurement of 5-HT_2 receptor sites (Frost, 1990).

Neuroendocrine challenge tests

This involves the measurement of changes in anterior pituitary hormone in response to challenge with 5-HT drugs. The hormones usually measured are prolactin (PRL), adrenocorticotrophin hormone (ACTH), and growth hormone (GH) (Tuomisto and Mannisto, 1985). Thus, if a standardised and selective 5-HT pharmacologic challenge of 5-HT pathways is employed, the size of the resulting hormonal response provides an index of the functional state of the 5-HT pathways mediating that endocrine response (Cowen et al, 1992).

Peripheral measures

Platelet 5-HT. Measures of platelet 5-HT uptake sites offer a model for the functional status of central 5-HT neurones. The human platelet expresses a functional 5-HT receptor which resembles the 5-HT_2 subtype (Geaney et al, 1984). It is controversial, however, whether platelet and 5-HT neuronal function can be equated. For example, a recent study of central 5-HT neurone lesioning in rats demonstrated profound changes in brain 5-HT levels and receptor binding, but no effect on platelet paroxetine binding (Moret and Briley, 1991). The suggestion from these findings is that peripheral mechanisms may be responsible for platelet 5-HT abnormalities found in depressed patients.

Plasma Tryptophan. This measure is discussed in section 1.1.2. It is regarded that levels of plasma tryptophan and the ratio of tryptophan to other LNAA's determine uptake into

the brain and hence, determine the synthesis of brain 5-HT. In animals it is clearly established that increasing tryptophan availability to the brain increases 5-HT metabolism (Gibbons et al, 1979), while tryptophan depletion produces the converse effect (Biggio et al, 1974).

1.2 THE ROLE OF 5-HT IN PSYCHIATRIC DISORDERS

5-HT has been implicated in a wide range of psychiatric disorders, more so than any other single neurotransmitter. As can be seen from the neuroanatomical structure of 5-HT pathways, central 5-HT has an important role in the regulation of a wide variety of neurobiological systems, and thereby human behaviour. From this premise it has been hypothesised that abnormalities in brain 5-HT may be implicated in various psychiatric disorders. A brief review of the main findings in this area of research will now be given. The role of 5-HT in eating disorders, a central theme of the present thesis, will be considered separately (section 1.3.5).

1.2.1 Affective disorder

Much research has centred on the role of 5-HT in depression. The most influential theory is the indoleamine hypothesis of depression. This was put forward in the early 1960s, incorporating three independent pieces of evidence suggesting that an abnormality of brain 5-HT function is central to the aetiology of affective illness (Coppen, 1967). Firstly, the antihypertensive agent reserpine which causes depletion of 5-HT and catecholamines, was associated with the development of 'depressive' symptomatology in some patients. Secondly, tricyclic antidepressants (TCAs) which are effective in the treatment of affective

disorders, inhibit the uptake of 5-HT. Finally, monoamine oxidase inhibitors (MAOIs), which inhibit the metabolism of 5-HT, are effective antidepressants.

Because of the complex nature of central 5-HT systems, the absence of a satisfactory animal model of depression, and difficulties in measuring central 5-HT function in man, the role of 5-HT in the aetiology of depression remains unclear. However, the past three decades has seen intense research in this area, and much evidence now exists to support a major role for 5-HT in the aetiology and treatment of depression.

5-HT receptor and 5-HT uptake studies. There is increasing evidence that in both suicide victims and patients dying while depressed, the density of cortical 5-HT_2 receptors is increased (Mann et al, 1986; Arora and Meltzer, 1989a; Yates et al, 1990). There are, however, negative studies in this area (Cooper et al, 1986; Cheetham et al, 1988). Similarly, while some authors have reported increased 5-HT_2 receptor binding on blood platelets in depressed patients (Arora and Meltzer, 1989b; Pandey et al, 1990), this abnormality is not consistently found. There is more agreement that platelet 5-HT uptake is lowered in depression (Healy and Leonard, 1987), but a similar claim for a reduction in platelet imipramine binding, which measures a site closely related to the 5-HT uptake mechanism, has not been substantiated (Briley et al, 1980; WHO, 1990).

Neuroendocrine studies. More consistent evidence for an abnormality in 5-HT function in depression comes from studies of 5-HT-mediated neuroendocrine responses. This is most apparent with regard to drugs that increase brain 5-HT function through precursor loading or facilitation of 5-HT release. Thus, the prolactin response to tryptophan (Heninger et al, 1984; Cowen and Charig, 1987; Deakin et al, 1990), the 5-HT reuptake

inhibitor clomipramine (Lopez-Ibor et al, 1989; Olden et al, 1990), and the 5-HT releasing agent fenfluramine (Siever et al, 1984; Mitchell and Smythe, 1990), is impaired in groups of drug-free depressed patients.

Plasma tryptophan. Plasma total and free tryptophan, and the ratio of plasma tryptophan to LNAAs have been measured in depressed patients. Decreased concentrations of tryptophan have been reported (DeMyer et al, 1981; Joseph et al, 1984; Maes et al, 1987). However, normal concentrations have also been reported (Riley and Shaw, 1976; Moller et al, 1979). Plasma total tryptophan and the ratio of tryptophan:BCAAs is decreased in depressed patients with melancholia (Anderson et al, 1990a; Maes et al, 1987; Cowen et al, 1989). It has been argued that these reductions may be a result of weight loss rather than the depression itself (Cowen and Anderson, 1991; Anderson, 1990a).

Manipulations of central 5-HT function affecting mood

Whilst evidence for a primary deficiency in 5-HT in depression remains controversial, a role for 5-HT in the aetiology and treatment of affective disorders is nevertheless supported by the effects of treatments which enhance or decrease central 5-HT function on mood.

Treatments which decrease 5-HT function. Observations that some patients treated with the monoamine depleting agent, reserpine, developed depressive symptomatology (Fries, 1954) were perhaps the first indication that decreasing central 5-HT function is associated with a lowering of mood. It remains unclear whether the 5-HT synthesis inhibitor, para-chlorophenylalanine (PCPA) also produces affective symptoms in non-depressed subjects, but it has clearly been shown to reverse the antidepressant effects of imipramine and

tranylcypromine (Shopsin et al, 1976).

Young et al (1985) adopted a relatively moderate manipulation of central 5-HT by tryptophan depletion. This manipulation has been adopted in the present thesis, and is outlined in detail in section 2.1. Young et al demonstrated a lowering of mood in healthy subjects, and Delgado et al (1990) have subsequently demonstrated a rapid return of depressive symptomatology in a group of patients remitted but maintained on antidepressant medication. These findings appear to confirm those of Shopsin et al (1976).

Treatments which enhance 5-HT function. The finding that TCAs inhibit the uptake of 5-HT and thereby presumably increase its synaptic concentration and 5-HT transmission, provided initial support for the indoleamine hypothesis of depression (Coppen, 1967). The effects of TCAs are ascribed to their inhibition of 5-HT uptake (as opposed to uptake of NA) in the light of studies demonstrating that co-administration of tryptophan may increase th antidepressant efficacy of these drugs (see Paykel and Hale, 1986). Whether, however, tryptophan administered alone represents an effective antidepressant treatment is still disputed (Byerly and Risch, 1985).

MAOI therapy increases the functional activity of several monoamines by inhibiting monoamine oxidase which catalyses the breakdown of monoamines including 5-HT. The clinical activity of MAOIs can be potentiated by administration of tryptophan (Coppen et al, 1963) suggesting that increases in brain 5-HT function may mediate its therapeutic action. This hypothesis receives support from the ability of PCPA and acute dietary tryptophan restriction to offset the antidepressant action of MAOIs.

Recent studies have examined the effects of increasing synaptic 5-HT availability using selective 5-HT uptake inhibitors. These compounds have been shown to be effective antidepressant compounds (Boyer and Feighner, 1991).

1.2.2 **Obsessive-compulsive disorder**

There is strong evidence that obsessive-compulsive disorder (OCD) is associated with a dysregulation of 5-HT function (Montgomery and Findberg, 1989). The main evidence for this has come from placebo controlled clinical treatment studies that have demonstrated the efficacy of selective 5-HT pharmacological interventions such as fluvoxamine and clomipramine over placebo (Thoren et al, 1980; Goodman et al, 1989). However, a direct 5-HT agonist, m-chlorophenylpiperazine (mCPP), counter to expectation results in an exacerbation of Obsessive-compulsive symptoms (Zohar et al, 1990). Furthermore, the beneficial action of clomipramine appears to be associated with blunting of the 5-HT response when compared with pretreatment baseline functioning (Zohar et al, 1990). A separate body of evidence comes from peripheral markers of 5-HT function. For example, improvement in OCD has been correlated with reductions in platelet 5-HT concentrations, and increases in levels of CSF 5-HIAA compared to healthy controls (Barr et al, 1992). However, these findings do not appear to be robust and it may be that only certain subtypes of OCD are associated with low 5-HT activity.

1.2.3 **Generalised anxiety and panic disorders**

A smaller number of studies have been carried out to examine the role of 5-HT in generalised anxiety and panic disorder. Much research has focused on models of

noradrenergic dysfunction, but there is now preclinical evidence that 5-HT dysfunction may be related to specific types of anxiety. It has been postulated that specific neuroanatomical sites exist for a serotonergic regulation of anxiety (Charney et al, 1990). This theory is supported by the antianxiety properties of selective 5-HT agonists such as buspirone and gepirone which act selectively at 5-HT_{1A} receptor sites (Kehne et al, 1988). There is a high density of this receptor type in the hippocampus, an important structure in the limbic system (Charney et al, 1990). Findings from clinical trials lend support to the preclinical investigations; for example, administration of MCPP to panic disorder patients results in an increase in anxiety and panic attacks compared with controls irrespective of the presence or absence of concurrent major depression (Kahn et al, 1988). Studies of platelet 5-HT function, however, appear to be equivocal with one study reporting higher levels of platelet 5-HT uptake compared with controls (Norman et al, 1986), and a second study reporting no significant differences (Balon et al, 1987). A number of 5-HT reuptake-inhibiting drugs have been shown to have antipanic properties in placebo-controlled studies (Charney et al, 1990 for review), although, as this review mentions, these reuptake inhibitors may be exerting their therapeutic effects via other neurotransmitter systems such as noradrenergic pathways. In conclusion, it seems that there is little consistent evidence to suggest a specific 5-HT abnormality in panic and GAD, but altering 5-HT function can have profound effects on the symptomatology of these syndromes.

The above summaries indicate the areas of research which have received the most attention in terms of the role of 5-HT in psychiatric disorder; this list is, however, by no means exhaustive. Important contributions have been made in understanding the role of 5-HT function in aggressive behaviour and suicide (review Markku et al, 1992); alcoholism (review Roy et al, 1990); sleep disorders (review Sharpley and Cowen, in press);

schizophrenia (review Csernansky et al, 1990); autism (review McBride et al, 1990); and alzheimer's dementia (review Gottfries et al, 1991).

In conclusion, it is evident that disturbances of 5-HT function may be of importance in the aetiology of many psychiatric disorders despite the diversity of behavioural syndromes which these disorders encompass. Simple conceptualisations of these disorders as either due to hyper or hyposerotonergic function are now regarded as largely inadequate. 5-HT pathways are distinct but widely distributed, and it is therefore not surprising that central 5-HT pathways are involved in many complex behaviours. Current research is attempting to further differentiate the role of 5-HT in the aetiology of these disorders by examining the actions of selective drugs on 5-HT receptor subtypes. The functional role of 5-HT_{1A} receptor in anxiety serves to illustrate the importance of investigating the effects of selective receptor subtypes. This type of evaluation will lead to a better understanding of the specific abnormalities in central 5-HT function. It is also true that the study of a single neurotransmitter is of limited use in understanding a particular disorder. It is very likely that other neurotransmitters such as dopamine and noradrenaline are important in, for example, depression (Meltzer and Lowy, 1987), and anxiety (Charney et al, 1990).

1.3 AN OVERVIEW OF THE EATING DISORDERS

The studies outlined in this thesis aim to explore possible psychobiological mechanisms in abnormal eating, which may have relevance to clinical eating disorders. These studies have largely been carried out in healthy female populations, with the exception of one study carried out in a population of women who have recovered from bulimia nervosa. Thus, the

studies aim to explore how normal mechanisms may become perturbed, and result in abnormal behaviour. In order to put this work in context, I will summarise the two disorders which are being considered in this thesis, and give an overview of competing theories in this field. This will be concluded with a review of the role of 5-HT specifically in anorexia and bulimia nervosa (section 1.3.5).

1.3.1 CLASSIFICATION, HISTORY, CLINICAL FEATURES, AND EPIDEMIOLOGY OF EATING DISORDERS

Eating disorders are classified by the American Psychiatric Association (ASA) in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) as gross disturbances in eating behaviour. This includes anorexia nervosa, bulimia nervosa, pica, and rumination disorder of infancy. Simple obesity is not regarded as a psychological disorder unless there is specific evidence that psychological factors are important in the aetiology or course of any particular case. Pica and rumination disorder appear not to be related to anorexia and bulimia, and are not considered in this thesis.

Diagnostic criteria for anorexia and bulimia as specified in the American Psychiatric Association (ASA) in DSM-III-R are set out below.

Anorexia Nervosa

A Refusal to maintain body weight over a minimal normal weight for age and height, eg, weight loss leading to maintenance of body weight 15% below that expected; or failure to make expected weight gain during period of growth, leading to body

weight 15% below that expected.

B Intense fear of gaining weight or becoming fat, even though underweight.

C Disturbance in the way in which one's body weight, size, or shape is experienced, eg, the person claims to 'feel fat' even when emaciated, believes that one area of the body is 'too fat' even when obviously underweight.

D In females, absence of at least three consecutive menstrual cycles when otherwise expected to occur (primary or secondary amenorrhoea). (A woman is considered to have amenorrhoea if her periods occur only following hormone, eg, oestrogen, administration).

Bulimia Nervosa

A Recurrent episodes of binge eating (rapid consumption of a large amount of food in a discrete period of time).

B A feeling of lack of control over eating behaviour during the eating binges.

C The person regularly engages in either self-induced vomiting, use of laxatives or diuretics, strict dieting or fasting, or vigorous exercise in order to prevent weight gain.

D A minimum average of two binge eating episodes a week for at least three months.

E Persistent overconcern with body shape and weight.

(From ASA, DSM-III-R, 1987).

It is recognised however, that there is a large overlap of symptoms between these two disorders, and it has been suggested that the proposed update of this classification system (DSM-IV) should contain separate subtypes of anorexia nervosa, that is, of a bulimic type and a nonbulimic type. Furthermore, it has been argued that a separate Binge Eating Disorder should be included. The lack of stability in the classification criteria is a reflection of the difficulty of classifying these disorders as completely separate illnesses. There are to a large extent overlapping features, and it has been argued that they may indeed be variants of one disorder with a shared core psychopathology.

Clinical Features

The essential features of this disorder centre around a refusal to maintain body weight, over a minimal normal weight for height, consequent upon an intense fear of gaining weight or becoming fat. This is usually accompanied by a distorted body image manifested by patients stating that they "feel fat" although underweight or even severely emaciated. In women, amenorrhoea is also present (Williams, 1987). A preoccupation with body shape and dissatisfaction with some aspect of their physical appearance is usual. The condition usually begins in adolescence and is seen predominantly in females (Fairburn et al, 1983).

Low body weight is usually accomplished by severe food restriction despite normal feelings of hunger and a preoccupation with food. Excessive exercise and laxative abuse are common as is the use of self-induced vomiting, with some studies reporting a prevalence rate of concomitant problems with bulimia amongst 40-50% of diagnosed anorectic patients (Mitchell et al, 1990). Chronic starvation results in a number of bodily changes such as shrinking of the stomach, the appearance of lanugo hair on the face, back and limbs and low pulse rate, low blood pressure and cold hands and feet and sensitivity to cold. A low basal metabolic rate and low circulating sex steroid hormone levels indicate the presence of endocrine abnormalities (Sharp et al, 1993).

Affective symptoms, especially depression and mood lability are common features and are often accompanied by social withdrawal and a lack of sexual interest (Fairburn et al, 1983).

Historical Background

From an historical perspective it is clear that the eating disorders have changed over time. Anorexia nervosa is regarded as a relatively recent illness, but it existed as far back as the 13th century to our knowledge; the first well documented case is that of Princess Margaret of Hungary, who was born in 1245 and was canonised not long after her death. Her life is very well documented in the Vatican records. From these documents, it can be ascertained that she exhibited many of the key symptoms of anorexia including a refusal to maintain body weight, continual fasting (with non-religious motivation), and a preoccupation with food, etc. (See Halmi, 1981 for a detailed description). There are many more recorded examples of anorexic cases throughout history, and it therefore seems likely that the disorder has been in existence for a considerable time.

Anorexia only emerged as a new disease in the 1870's despite the fact that historical research has revealed examples of cases throughout recorded history (Brunberg, 1988). Brunberg argues that the emergence of anorexia as a specific disorder is a consequence of medicine's increasing empiricism and sophistication in its aim to distinguish one ailment from another; at this time there was an intellectual push for new nosologies and differential diagnosis. However, generally speaking, at this time anorexia was regarded as a symptom of disease rather that one in its own right. It was William Withey Gull in the late 19th century who first conceived of anorexia as a coherent disease entity, unrelated to organic disease or any of the various classes of 'insanity' which existed at this time.

Epidemiology

From the turn of the century onwards, the incidence of anorexia nervosa in industrialised countries has risen, such that it has been described as an epidemic in recent years. Epidemiological research spans back over the last 50 years, and we must rely on an examination of this time period in order to assess rates of incidence, demographic data, and information about the clinical population. Several studies report an increase of incidence of anorexia nervosa during the last few decades (eg Willi and Grossmann, 1983; Kendell et al, 1973). Willi and Grossmann report rates of 0.38/100,000 from 1956-1958 and 1.12/100,000 for 1973-1975. A comparative figure for a UK population is higher than this; for example Szmulker et al (1986) report rates of 4.06/100,000 population for 1978-1982. A follow-up study carried out by Willi and Grossmann (1990) reports that in recent years there has been no significant increase in incidence of anorexia in the same population. If this trend generalises to other populations, then the rise in incidence may have reached a plateau. This however, may be a result of more recent increases in the incidence of bulimia

nervosa. It is important to note that these figures reflect numbers of individuals seeking treatment and thus, general population rates are likely to be significantly higher. Another factor to be considered is that GPs and community workers have become much more skilled at diagnosis of eating disorders as awareness of the problem has grown, and this may have created an apparent increase in incidence rates with increase in referral for treatment. There are also large variations in diagnostic criteria over time and between populations that may account for some of these differences.

It has been convincingly argued that anorexia is more common amongst adolescent girls from middle class backgrounds and certainly, older studies report this bias in their data (eg Bruch, 1965). However, more recent studies reveal that the social bias may no longer exist to such an extent (Szmulker et al. 1986). One explanation for this is that anorexia is the product of culturally determined attitudes and behaviour patterns, which may have originally belonged to middle class families, but have now become much more part of general societal attitudes (Kendell et al, 1973).

Bulimia Nervosa - Clinical Features, Historical Background, and Epidemiology

Clinical Features

Central to the disorder of bulimia nervosa are recurrent episodes of binge eating, defined as the rapid consumption of large amounts of food within a discrete period of time, with an associated feeling of loss of control over eating during the episodes (Williams, 1987). Weight gain is avoided by the use of either self-induced vomiting, laxative or diuretic

abuse, strict dieting or fasting between binges or by the use of vigorous exercise. The frequency of binge-eating and vomiting varies, but can occur ten times or more each day (Fairburn et al, 1983). A persistent overconcern with body shape and weight is also present (Williams, 1987).

Patients with bulimia are usually of normal weight although a subgroup tend to be slightly overweight (Russell, 1979). The syndrome is seen predominantly in females with only 10-15% of cases affecting males (Carlat et al, 1991). The age of onset is usually in late adolescence or early adulthood. Unlike anorexia nervosa, amenorrhoea is not present although menstrual irregularities have been reported in up to 50% of cases (Fairburn et al, 1983) and patients are more likely to be sexually active. Depressive features including low self esteem and feelings of worthlessness are common, as are symptoms of anxiety which can be severe and disabling (Cooper et al, 1986). In some patients, impulsive behaviours such as stealing, alcohol or drug abuse and sexual promiscuity are also seen (Fairburn et al, 1983).

Physical complications may result from bulimia, usually from self-induced vomiting or purging behaviours and may become life-threatening. These include electrolyte imbalances which may induce cardiac arrhythmias, urinary infections and renal failure, epileptic seizures, swollen salivary glands, dental decay and widely fluctuating weight levels (Russell, 1979).

Historical Background

Bulimia nervosa has been recognised as a specific disorder for a very short time. In 1979

Gerald Russell put forward a theoretical model which postulated that there was a distinct subset of symptoms occurring in some anorexic cases which were partially independent of true anorexia. He emphasized the symptoms of overeating followed by self-induced vomiting or purging, with a morbid fear of becoming fat. Although Russell suggested that it was perhaps premature to consider the disorder he described in terms of a distinct syndrome, this marks the beginning of a nosological change in the definition of the eating disorders, to include bulimia nervosa. Symptom clarification has subsequently been refined and added to his description with, in particular, a more precise definition of a 'binge'. DSM III contained a category entitled 'bulimia' which referred to binge eating specifically. However, DSM III-R contains a description of the full syndrome in which the three core features are described (ie binge eating , control of body weight, extreme concern of body weight and shape)

Despite the very modern nature of the disorder and the widely held assumption that the symptoms associated with it have only emerged during the last twenty years, the occurrence of episodic binge eating is certainly nothing new. Roman civilisations, renowned for their characteristic wealth and gluttony, reportedly indulged in excessive eating followed by induced vomiting. From a more clinical point of view, evidence can be assembled for the existence of binge eating over the last three centuries. Early 18th century medical dictionaries referred to binge eating under several headings such as 'fames canina' (hunger of a dog), 'bolimus' (hunger of an ox), and more specifically, 'caninus appetitus' which included eating followed by vomiting (see Pope and Hudson, 1984 for review). In 1797 the Encyclopedia Britannica included the term 'bulimy' which was described as a disease 'in which the patient is affected with an insatiable and perpetual desire of eating' (Jansen, 1990). In the late 19th century Gull (1888) noted that binge eating could be present as part

of the anorexia nervosa syndrome. Slowly binge eating was seen not as an isolated phenomenon but as part of a wider pathology characterised by dieting behaviour and an abhorrence of the body.

Epidemiological studies of bulimia nervosa are limited by the very short time span over which the syndrome has existed. Even within this 25 year period, the diagnostic criteria for the disorder have changed complicating the comparison of prevalence rates from various studies.

Halmi (1981) and Hart (1985) report that up to 60% of American female college students experience episodes of binge eating, and that 5-13% meet DSM III criteria for bulimia nervosa. Using a self-report questionnaire design, a UK study (Cooper, 1983) report that a female population (attending a family planning clinic) have a rate of 20.9% binge eating with 2.9% using self-induced vomiting to control their weight. It was estimated that only 1.9% of this population met criteria for DSM III diagnosis. A further study reports life time prevalence rates of 1.9% for females (Bushnell, 1990). However, many of these studies suffer shortcomings arising from their self-report methodologies which tend to produce higher rates of prevalence than interview studies. Interview methodologies in this field have been criticised for adopting respondent-based interviews which share many of the same limitations of the self report questionnaires (Fairburn et al, in press).

Attempts have been made to establish the stability of the prevalence rates over time. It would appear from the literature that these rates are increasing at a very fast rate, (see Fairburn et al, in press, for review), and have even been considered as of epidemic proportion. Studies carried out in America and New Zealand report a three to six-fold

increase in the prevalence of DSM III bulimia nervosa within the last decade (Hall, 1991; Pyle, 1986). However, not all studies have reported increasing prevalence rates; a replication of the Cooper et al (1983) study four years later reported very similar results with a 1.8% prevalence rate for bulimia nervosa compared with 1.9% (Cooper, 1987).

In conclusion, it is very difficult to assess actual prevalence rates of bulimia nervosa, as the reported rates vary enormously between and within populations. This reflects a problem with diagnostic criteria, biases in population samples, variations in data collection methodologies, and of course, the possibility (as yet unproven) that there are genuine differences between populations in the prevalence rates of this disorder.

1.3.2 AETIOLOGICAL THEORIES OF THE EATING DISORDERS

In attempts to understand the underlying mechanisms of these disorders, psychology and clinical medicine have produced numerous theories regarding the causal factors important in their development. The principal theories with regard to anorexia and bulimia nervosa will be outlined in sections 1.3.2.1 and 1.3.2.2.

1.3.2.1 Aetiological theories of anorexia nervosa

While the fundamental causes underlying anorexia nervosa are not fully understood, the disorder is now usually viewed as arising from a complex interaction of biological, psychological and social factors. Fairburn (1983) has suggested that these factors may function by imparting vulnerability, or by acting as precipitants to, or perpetuators of the illness.

Early studies were heavily influenced by traditional psycho-analytical teaching, viewing the disorder as a defense against fantasies of impregnation. This idea was later replaced by that of Bruch (1962), who saw the disorder as arising from a disturbance of body image, seen alongside the anorectic's inability to interpret her own bodily sensations and paralysed by an overwhelming sense of ineffectiveness and lack of autonomy. Slade (1973) went on to describe an objective technique for determining bodily perception and used it to demonstrate that anorectic patients markedly overestimated their own body width.

Crisp (1976), described the core pathology as the development of a weight phobia "hinging on and pivoting round puberty". The central phobia was not seen as being directed towards an excess of weight but towards a "normal (functioning) adolescent weight". Crisp felt that for some individuals, fearful of adolescence, an association developed between the normal weight gain of puberty and the psychosexual and other maturational tasks expected by these changes. Carbohydrate starvation acted to halt the onset of adolescence and provided a means of returning to the safe and familiar state of childhood.

Personality theorists have generated much research in this area.

These studies are based mainly upon the analysis of detailed clinical information and case study reports, and the results have, at best, been conflicting and must be interpreted in the light of their serious methodological limitations (Smart et al, 1976). Some authors have failed to find a uniform personality structure, while others have emphasised traits such as obsessionality, conformity and goodness (Bruch, 1962, Crisp, 1976). Even those studies that have used objective personality assessments require careful interpretation because of methodological problems such as the use of nonvalidated scales and single assessments carried out at the time of illness. Overall, the results have continued to provide

inconclusive evidence, although individual studies have reported personality profiles suggesting high neuroticism and introversion (Smart et al, 1976).

In contrast, theory examining family dynamics has drawn attention to the possibility that factors existing outside the individual may influence the onset of the disorder. Minuchin (1975), in his development of an open systems family model outlined three conditions he believed to be necessary for the development and maintenance of severe psychosomatic disorders (including anorexia nervosa) in children. In addition to a child's physiological vulnerability, he described a type of family organisation that "encourages somatization". In particular, four characteristics i.e. enmeshment, overprotectiveness, rigidity and a lack of conflict resolution were said to characterise the way in which families of anorectics operated. The final condition proposed in this family model was the tendency by the family to use the ill child to avoid family conflict.

In a study of 56 families of anorectic patients, Kalucy (1977) identified another set of factors, which he called "weight pathology". "Weight pathology" described "deviations in weight, shape, eating behaviour and activity" which he reported to be present in 40% of anorectic families. However, in later research, designed to test Kalucy's hypothesis, Hall (1986) was unable to demonstrate any significant differences in either weight history or attitudes towards weight-related matters between 58 mothers of anorectic patients and a matched control group of 204 mothers of New Zealand schoolgirls. Garfinkel (1983) also failed to find differences between his parents of patients and control groups with respect to weight, eating attitudes test scores (Garner et al, 1979) and on measures of body satisfaction or idealization of thinness.

Many of these earlier hypotheses, which are heavily influenced by psycho-analytical theory, and more recently, theories of personality and family dynamics, have intuitive appeal. However, many are based on case reports and eschew the possibility of rigorous scientific examination. These theories can be regarded as mainly descriptive in nature, with little scientific evidence to support them.

In the cognitive-behavioural model of anorexia nervosa, dysfunctional thoughts and attitudes are seen as central to the maintenance of the disorder. Most of these cognitive distortions revolve around a core belief that thinness is not only highly desirable but comes to represent the sole measure of success. Weight loss, often positively remarked upon by others in the early stages of dieting, is said to become a positive self-reinforcer in itself as patients experience the pleasure of successful weight loss and develop a sense of control over their lives through the discipline dieting represent (Garner et al, 1982). As anxiety about eating increases, an intense fear of food and weight gain develops, which is further reinforced in a negative way by the avoidance of feared situations (Garner et al, 1982). The importance of cognitive factors in the maintenance of these avoidant behaviours has been emphasised by Bandura (1978) who stated that "behaviour is so powerfully controlled by bizarre internal contingencies that neither the beliefs nor the accompanying actions are much affected even by extremely punishing environmental consequences." Dysfunctional thoughts are addressed in cognitive-behaviour therapy which seeks to question the validity of these thoughts, and their role in maintaining the disorder. However, in most cases of anorexia this would be carried out in the context of an eclectic treatment programme, and it is therefore difficult to assess the outcome of such treatment. Moreover, this type of theory is of limited value in an analysis of causality, but rather offers an explanation of maintaining mechanisms.

Evidence for genetic contribution to the aetiology of anorexia nervosa comes from family and twin studies. The finding that a disorder occurs with increased prevalence within families of probands compared to the general populations is consistent with either a genetic or environmental mode of transmission. However, given that twins raised together share the same cultural and environmental influences, the presence of a higher concordance rate amongst monozygotic (MZ) twins (who share a higher percentage of the same genes) verses dizygotic twins (DZ) is best explained by genetic rather than cultural or environmental factors (Holland et al, 1988).

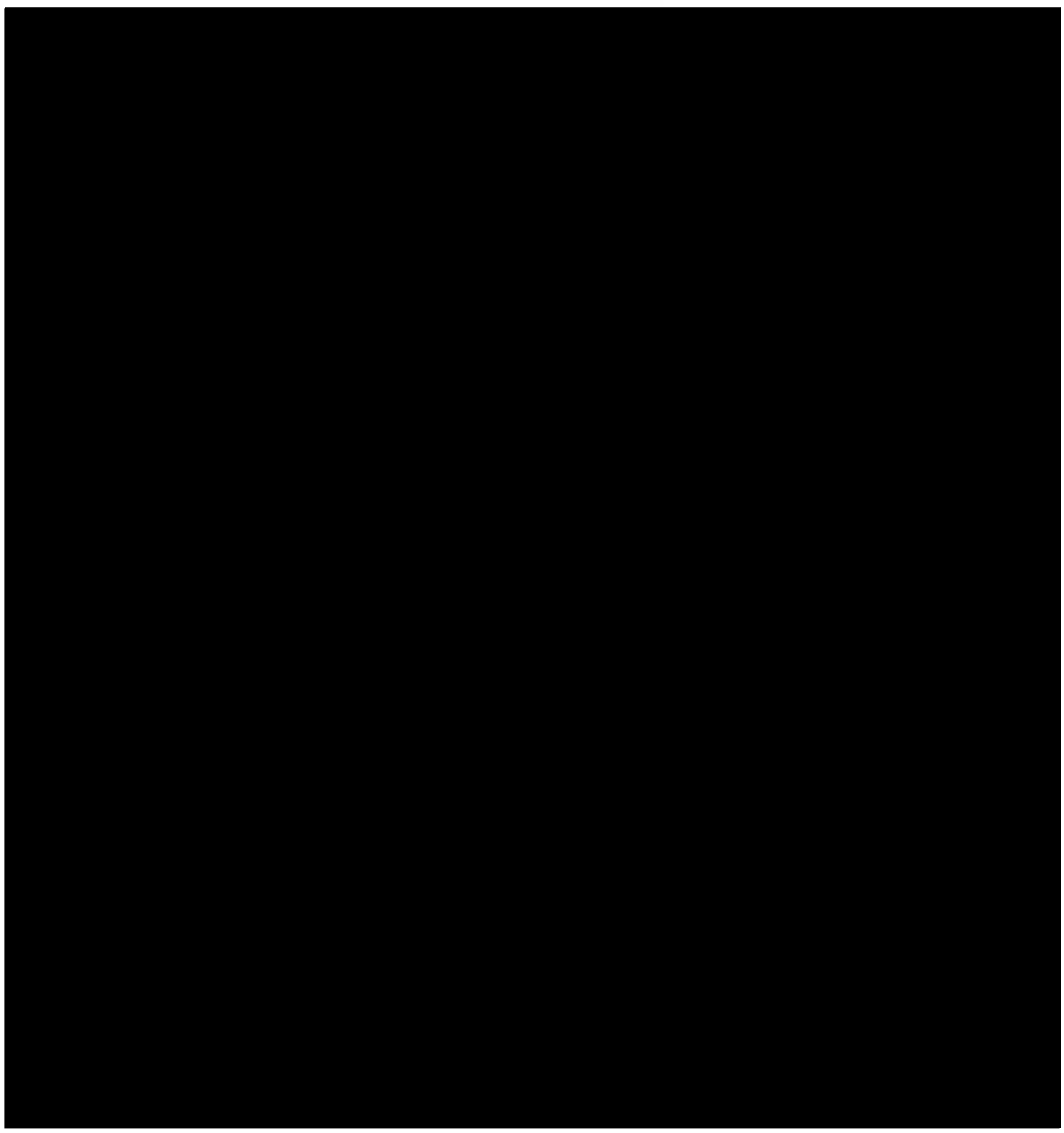
Family studies have consistently demonstrated an increased prevalence rate of anorexia nervosa amongst first degree relatives of anorectic patients. Theander 1770) reported a 6.6% sibling risk for the disorder in his study of 94 anorectic probands while Crisp (1980) found that 7% of siblings had either had or were still suffering from anorexia nervosa. Furthermore, 14% of the mothers and 9% of the fathers were also reported to have histories consistent with the disorder, although some of these diagnoses were described as "marginal". Holland et al (1988), reporting only on female first-degree relatives found a rate of nearly 5%. Other studies have reported an increased risk of both eating and affective disorders amongst first degree relatives (Gershon et al, 1984; Hudson et al, 1983a).

A number of twin studies have now also been completed. Earlier studies have been criticised on the basis of inadequate diagnostic clarification and inaccurate determination of zygosity. More recently Holland (1984) reported that of 30 female twin pairs, 55% of the MZ twins were concordant for anorexia nervosa compared to only 7% of the DZ pairs, although the question of selection bias with this particular group of patients was raised. A Follow-up study, designed to minimise this problem was later completed and reported a

56% concordance rate for anorexia nervosa in twenty-five MZ female twins compared to 5% of DZ pairs. Thus, whilst many of these studies contain methodological weaknesses, there is a clear suggestion of a genetic predisposition to the development of this disorder.

The influence of socio-cultural factors in the aetiology of anorexia nervosa has been suggested by several lines of research. From very early studies an overrepresentation in upper social classes has been noted (Kendell et al, 1973, Szmukler et al, 1986, Crisp et al, 1976) and to some extent the disorder appears to be confined to areas where a "western" style culture predominates. It has been suggested that the relatively recent shift in the idealized female shape from a curved female figure to a thin, lean look has placed increasing pressure upon western women to conform and may have contributed to the rising incidence of anorexia nervosa (Kendell et al, 1973, Szmukler et al, 1986, Garner et al, 1980, Willi, 1983). In support of this theory, Garner et al (1980) cite data from magazine centrefold and Miss American Pageant contestants which demonstrate a significant trend towards a thinner ideal. The average weight of the Miss America Pageant contestant has fallen over twenty pounds in the past thirty years. They further point out that these changes have occurred against a trend towards increasing standard body weight and size for North American women. The impact of this pressure to conform is said to be reflected in the high levels of reported body weight and shape dissatisfaction, and the fact that dieting behaviour is endemic amongst young females in our culture today (Whitaker et al, 1989, Johnson-Sabine et al, 1988). Fig 1.3 demonstrates the changing perceptions of the ideal body weight over time. These two illustrations are taken from textbooks, and it is surprising how closely they conform to cultural norms of their time. These studies offer interesting insights into societal pressure, but are largely correlational in design, and thus offer a limited explanation of causal factors.

Fig 1.3



Changing fashions in women's figures. Illustration a) is from an obstetrical textbook printed in England in 1840, and b) is from a textbook printed in 1981. (From Abraham and Llewellyn-Jones, 1987).

In an attempt to examine this question of causality, Garner et al (1980) examined a population of professional dance students and fashion models, who by their career choice it could be argued, would face intense pressure to maintain body weight and shape close to the current accepted ideal. 7% of the modelling and 6.5% of the dance group met strict criteria for anorexia nervosa with all but one case in the dance group developing the disorder while studying dance. Furthermore, within the dance group, the risk for anorexia was higher amongst those students in programmes in which there was greater pressure to succeed as professionals. The authors argue that "these data suggest that both pressures to be slim and achievement expectations are risk factors in the development of anorexia nervosa", although they do not consider the fact that it would be difficult to separate out these two factors as for these students, achievement would depend upon slimness.

Neurobiological theory stems mainly from clinical studies of neurotransmitter and neuroendocrine function, thus leading to speculation about their role in the aetiology of the disorder. Theories regarding the role of 5-HT in eating disorders will be reviewed in section 1.3.3.

There are consistent findings of abnormalities of hypothalamic-pituitary and neurotransmitter function in acutely ill patients (Garfinkel et al, 1975; Gerner et al, 1981; Gwirtsman et al, 1989a, Halmi et al, 1987; Kaye et al, 1990). While earlier theories such as anterior pituitary insufficiency have now been largely discarded, debate continues over the significance of other neuroendocrine abnormalities. It is not clear whether these abnormalities are intrinsic to the disorder or a consequence of the illness (Halmi et al, 1987; Gwirtsman et al, 1989a). To date almost all research in this area has been carried out on acutely ill patients, where it is difficult to disentangle the effects of starvation, weight loss,

purging, and, in the case of recently 'recovered' anorectics, the effects of refeeding. Research findings therefore need to be interpreted in this light.

Most of the endocrine abnormalities observed in anorexic patients are now thought to reflect starvation, as most of these indices return to normal upon weight restoration (Halmi et al, 1987). These include hypercortisolaemia, reduced gonadotrophin levels, low serum triiodothyronine (T_3) and delayed thyroid stimulating hormone (TSH) responses to thyroid releasing hormone (TRH), elevated basal growth hormone levels, and incomplete suppression of ACTH and cortisol by dexamethasone (Garfinkel et al, 1975; Gerner et al, 1981; Gwirtsman et al, 1989a; Halmi et al, 1987).

However, studies of neurotransmitter systems raise the possibility that abnormalities specific to anorexia nervosa may underlie the disorder, as evidence suggests that these abnormalities may persist with recovery. Evidence that the noradrenergic system may be involved comes partly from studies looking either directly at cerebral spinal fluid (CSF) levels of noradrenaline (NA) and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), and peripheral MHPG levels in plasma and urine. Acute illness is associated with lower levels of plasma and urine MHPG and CSF NA and MHPG, suggesting reduced NA turnover (Halmi et al, 1978; Halmi et al, 1987; Gerner et al, 1981). However, it has been reported that CSF NA levels in long-term weight recovery (approx 20 months) are 50% lower than controls, and it has been argued that this may be a trait marker for the disorder (Fava et al, 1989).

Neuroendocrine challenge and receptor binding studies of NA function similarly find normalisation with weight restoration in many cases. However, using a clonidine-induced GH response as a measure of postsynaptic sensitivity, it has been found that this response

is normal in low-weight anorectics, but blunted following weight restoration (Kaye et al, 1985).

Abnormalities of other neurotransmitter systems have also been postulated. Studies of dopamine metabolism have been largely inconsistent (Fava et al, 1989), but there is some evidence for alterations in CSF opioid activity in underweight anorectics compared to controls (Kaye et al, 1982). However, it is the 5-HT system that has been most implicated in the pathogenesis of the eating disorders; this literature will be reviewed in section 1.3.3.

1.3.2.2 Aetiological theories of bulimia nervosa

In common with anorexia nervosa, the true aetiology of bulimia nervosa remains unknown, although a complex interaction of biological, psychological and social factors have similarly been proposed.

Perhaps the most important predisposing factor so far identified is a past history of anorexia nervosa (Fairburn et al, 1983). While more than half of Russell's (1979) original group of bulimics had unequivocal past histories of anorexia nervosa (a rate now thought to reflect a unique referral population), a community study has suggested the true incidence to be much less than 50%, including highly doubtful cases (Pyle et al, 1981). Indeed, Mitchell et al (1985) found that only 4.3% of their 275 bulimic patients had been treated in the past for anorexia nervosa or low body weight. Nevertheless, these rates are much higher than those seen in the general population.

Even where a past history of frank anorexia nervosa has not been found, it is usual for a

period of food restriction or dieting to have preceded the onset of binge-eating (often in response to being slightly overweight), while the clinical picture frequently consists of alternate severe food restriction and compensatory binge-eating (Fairburn et al, 1983). Wardle et al (1981) and Polivy et al (1985) have proposed that an association exists between excessive dietary restraint (i.e. the conscious control of food intake in order to prevent weight gain or to ensure weight loss) and binge-eating. They have further argued that this association is causal (see section 1.4.2 for review). Although it is increasingly recognised that episodes of bingeing, food craving and difficulties with stopping eating once started are behaviours that occur in unrestrained eaters and are in themselves probably not abnormal, the frequency of these activities is much greater in highly restrained eaters (Wardle et al, 1981). Bingeing may represent a physiological response to short periods of starvation or to trying to maintain one's weight below a natural "set-point" for body weight which has been proposed by Nisbett (1972) to exist for us all.

Theories grounded in cognitive psychology regard the phenomenon of "counter-regulation" as an important mechanism leading to bingeing. Counter-regulation refers to the phenomena whereby restrained eaters will eat more following a large energy preload compared to a small energy preload, a response in direct contrast to that seen in unrestrained eaters and in contrast to what might be expected in purely physiological terms (Hibscher et al, 1977). The importance of the belief regarding the amount of calories consumed, rather than the actual energy load ingested, was further demonstrated by Wooley (1972) and it has been suggested that once the highly restrained eater thinks she has overeaten or exceeded her dietary allowance, she abandons all dietary constraints. A similar mechanism has been proposed to underlie binge-eating (see section 1.4.2 for review).

The importance of the patient's beliefs and attitudes to weight and shape are emphasised in the cognitive-behavioural model of bulimia nervosa. It is these attitudes that are seen as central to the maintenance of the disorder (Fairburn et al,1986) and must therefore be altered before lasting recovery can occur. Typical belief systems include a tendency to evaluate self-worth and success only in terms of slimness and personal attractiveness, a vehement dislike of fatness and a positive attitude to the maintenance of self-control (Fairburn et al, 1986). While Fairburn points out that such attitudes are widely held in western societies, it is the rigidity and extremity of the bulimic patient's beliefs (which are also seen as having great personal significance) that lead to dysfunctional styles of reasoning. Such thinking styles include "dichotomous thinking, overgeneralisation and errors of attribution" and through their emphasis on the extreme importance of weight and shape are thought to explain the maintenance of much of the patient's behaviour (Fairburn et al, 1986). The value of a cognitive-behavioural model can to some extent be evaluated by it's treatment success. A recent study has compared cognitive behavioural therapy with interpersonal therapy and behaviour therapy (Fairburn et al, 1988). It was concluded that at three month follow-up, cognitive-behaviour therapy and interpersonal therapy were equally effective on behavioural measures (with a 77-78% reduction in vomiting) compared with the less effective behaviour therapy. Studies on long-term outcome which would allow a better evaluation of lasting change, are still missing.

Some evidence now exists from family and twin studies for a genetic vulnerability in the development of the disorder, although methodological limitations outlined for the anorexia nervosa studies similarly apply. Nevertheless, in an uncontrolled family study by Hudson et al (1983a) the prevalence of bulimia amongst relatives of bulimic probands was found to be 3.4%, while a controlled study by Kassett et al (1989), who adopted a direct interview design, reports an increased risk of all eating disorders amongst relatives of bulimic

probands compared to relatives of normal controls. However, not all research groups have demonstrated this increased risk for family members (Hudson et al, 1987; Logue et al, 1989). To date, only a few twin studies have been published but these also support the possibility of a genetic predisposition, with reported concordance rates for bulimia in MZ and DZ twins ranging between 22.9%-83% and 0%-26.7% respectively (Logue et al; 1989, Fichter et al, 1990; Hsu et al, 1990; Kendler et al, 1991). Fichter's study also reported a higher concordance rate for any eating disorder in MZ twins compared to DZ twins.

A highly significant association has been found between bulimia nervosa and the affective disorders, especially depression, although the exact nature of the relationship remains uncertain (Russell, 1979; Hatsukami et al, 1984; Walters et al, 1992). While depressive symptoms are commonly seen in patients suffering from bulimia nervosa and many patients meet diagnostic criteria for affective disorder at some stage in their lives (Fichter et al, 1990; Mitchell et al, 1986; Pope et al, 1983), several studies have also reported higher rates of affective disorder amongst first-degree relatives of bulimic probands than amongst relatives of both psychiatric and normal controls (Hudson et al, 1983b; Kassett et al, 1989; Hudson et al, 1987; Logue et al, 1989). One study by Wilson et al (1987), looking at fifty-seven bulimic patients, suggested a correlation between the severity of the eating disorder and the degree of depression. These authors reported that the more severe the bulimia, "the more likely the bulimic was to be suffering from depression to a very high degree".

Other studies have reported higher than expected rates of substance abuse, both alcohol and drugs (Kassett et al, 1989) and obesity (Fairburn et al, in press) amongst relatives of bulimic probands and have suggested that these may also represent risk factors in the development of the disorder.

In keeping with the studies on anorexia nervosa, no consistent personality type has been found that is thought to predispose to the development of the disorder. However, patients suffering from bulimia nervosa share the anorectic's discontent with their body shape and weight, tend to have low self esteem and a significant subgroup are reported as having impulse-control difficulties (Fairburn et al, 1983). A high rate of childhood sexual experiences has been reported by women with eating disorders (Palmer et al, 1990). However, methodological problems existed with these early studies and later, better designed research has not always confirmed the initial findings, raising the question of whether sexual abuse is indeed a risk factor (Fairburn et al, in press).

Socio-cultural factors thought to be important for the large increase in cases of anorexia nervosa may well apply equally to bulimia, but this has received little attention to date.

Biological theories have considered neuroendocrine and neurotransmitter function in bulimia nervosa. The symptoms of amenorrhoea and oligomenorrhoea (Fairburn et al, 1984), and hormone abnormalities which all suggest disturbances of the hypothalamic-pituitary-adrenal axis (HPA), have led to interest in the possibility that a neuroendocrine disturbance may underlie the disorder or play a role in its maintenance (Kaye et al, 1991; Leibowitz et al, 1990). However, as with anorexia nervosa, research in this area is sparse and shares similar methodological limitations. These include the confounding effects of ongoing bingeing and purging, resulting malnutrition, and the presence of affective illness, and lack of studies on long term recovered patient populations. Despite these problems, however, some initial findings have been reported.

The most extensively studied system has been the HPA (Weltzin et al, 1991) which has

consistently demonstrated a failure to suppress cortisol following a dexamethasone suppression test (DST) in 20-67% of normal weight bulimic women (Gwirtsman et al, 1983, Mitchell, 1983). It is unclear whether this reflects the presence of affective symptoms, malnutrition and/or low weight (Gwirtsman et al, 1983), a failure of bulimic patients to absorb or metabolise dexamethasone (Walsh et al, 1987) or an intrinsic neurobiological abnormalities distinct from the above epiphenomena.

Other abnormalities of this system reported in normal weight, acutely ill bulimic patients include delayed or blunted TSH responses to TRH (Levy et al, 1988; Gwirtsman et al, 1983) and abnormal GH and cortisol responses to an intravenous glucose tolerance test (Coiro et al, 1992). Studies of hormone levels have been inconsistent with 24-hour cortisol secretion reported as either normal (Walsh et al, 1987; Weltzin et al, 1991) or elevated (Mortola et al, 1989). This inconsistency is also true for basal GH, PRL and ACTH levels (Weltzin et al, 1991; Mortola et al, 1989; Mitchell et al, 1983; Levy et al, 1988). Pituitary-adrenal function has been examined following one month abstinence of bingeing and vomiting (Gwirtsman et al, 1989a). Indices of this are normal in acutely ill bulimics, but CSF ACTH levels are significantly reduced after one month. However, a negative correlation of energy consumption and decrease in CSF ACTH suggest that this is a reflection of calorie restriction during this period (Gwirtsman et al, 1989b).

Neurotransmitter abnormalities have been widely reported in actively ill bulimics. NA alterations include reduced plasma and CSF NA levels (Kaye et al, 1990a; Kaye et al, 1990b). Both lymphocyte and mononuclear leucocyte ß-adrenoceptor activity has been reported as increased in some studies, but not all (Buckholtz et al, 1988; Lonati-Galligani et al, 1986). Reports of increased platelet alpha-2-adrenoceptor activity are similarly

inconsistent (Heufelder et al, 1985; Kaplan et al, 1989).

To date, little work has been carried out on other neurotransmitter systems in bulimia, with the exception of the 5-HT system which will be reviewed in section 1.3.3. Studies of dopamine function have reported inconsistent results with CSF levels of homovanillic acid (HVA) as normal (Kaye et al, 1990b) and reduced (Jimerson et al, 1992) in acutely ill patients. Evidence suggests that peptide neuromodulators such as cholecystokinin (CCK) and neuropeptides such as neuropeptide Y (NPY) are dysregulated in bulimia (Geracioti et al, 1988; Kaye et al, 1990c). These are of interest because of their role in regulating satiety and stimulating carbohydrate feeding respectively (Smith et al, 1985; Stanley et al, 1986). Impaired plasma CCK responses to food intake and post-prandial satiety have been reported (Geracioti et al, 1988), and CSF peptide YY concentrations are elevated after one month's abstinence from bingeing and purging compared to the acutely ill phase of the disorder (Kaye et al, 1990c).

In summary, it can be seen that evidence suggests a complex genetic vulnerability with associated affective disorder, and risk of substance addiction. Cognitive theory delineates mechanisms that may be causal, and have a role in the maintenance of the disorder. In addition, biological theories offer some evidence for neuroendocrine and neurotransmitter abnormality.

Thus, no one theory has taken precedence in explaining the aetiology of bulimia nervosa.

1.3.3 5-HT, ANOREXIA AND BULIMIA NERVOSA

The balance of pharmacological evidence to date, from both animal and human studies, supports the hypothesis that serotonergic dysfunction occurs in the eating disorders (see Leibowitz, 1990 for review). Research in this area has escalated over the past two decades, in particular, findings from basic animal work have been applied to human studies in an attempt to understand the exact nature of the serotonergic abnormalities.

1.3.3.1 5-HT and Anorexia Nervosa

Precursor and metabolite studies. Early studies looking at 5-HT function in anorexia nervosa examined peripheral concentrations of tryptophan and the major metabolite of 5-HT, 5-HTIAA, in urine and plasma. Such findings produced inconsistent results in the acutely ill patient. Coppen et al (1976) reported reduced free and bound plasma tryptophan levels, whilst Russell et al (1967) did not. A comparison of the tryptophan/LNAA ratio before and following short- and long-term recovery with controls also reported no differences (Kaye et al, 1984). These findings argued against the hypothesis of reduced plasma tryptophan availability to the brain for 5-HT synthesis in the acute phase of the illness. However, evidence from such a small number of studies is by no means conclusive.

CSF 5-HIAA concentrations have consistently been reported as low in malnourished and underweight anorectic patients (Gillberg et al, 1983; Kaye et al, 1984; Kaye et al, 1988a). However, with refeeding and short-term weight restoration (2 months), CSF 5-HIAA levels appear to normalise compared with controls (Kaye et al, 1988a; Gerner et al, 1984). Thus the reduced levels are thought to reflect starvation, as even partially refed patients with

minimal weight gain have been reported as having normal levels (Gerner et al, 1984). However, Kaye et al (1991) studied 5-HIAA levels in long-term weight restored patients who had maintained a stable weight above 85% average body weight (ABW) for at least 6 months. They report increased 5-HIAA concentrations in the CSF compared with controls. Although some methodological difficulties exist with this study such as studying a mixed restrictor/bulimic population, the inclusion of patients who continued to purge and binge and/or were still amenorrhoeic, it raises the possibility of increased brain 5-HT function as a trait marker in long-term recovered anorexia nervosa.

Some differences between restrictor and bulimic subgroups have also been demonstrated. After blocking active transport of 5-HIAA out of the CSF with intravenous infusion of probenecid, accumulated levels of the metabolite were consistently higher in the restrictor anorectic patients than the bulimic group. This was not related to length of recovery (Kaye et al, 1984). The hypothesis that restrictor anorectics have greater 5-HT activity is further supported by a treatment study using the 5-HT receptor antagonist cyproheptadine in which restrictor anorectics were found to respond significantly better than the bulimic subgroup (Halmi et al, 1986). This CSF 5-HIAA difference may reflect differences in weight and nutrition, membrane transport or other differences in central probenecid effects between the subgroups (Kaye et al, 1984), but, more excitingly, it may represent a trait marker for the two subgroups reflecting 5-HT turnover rates that are independent of starvation or other central effects (Brewerton et al, 1990). Indeed it has been suggested that greater serotonergic activity amongst restrictor anorectics may account for their more obsessional, ritualistic, perfectionistic and rigid personality traits which, it has been argued, persist even with long-term weight recovery and may contribute to the high relapse rates seen in this disorder (Kaye et al, 1991).

Platelet studies.

There are few studies of platelet 5-HT uptake and imipramine binding in anorexia nervosa, but there is a report of decreased imipramine binding in the acute phase of the illness (Weizman et al, 1986), suggesting reduced 5-HT activity. However, 5-HT uptake was not found to be different between anorectics and controls (Weizman et al, 1986; Zemishlany et al, 1987). Neither of these studies reported on bulimic versus restrictor subtypes.

Platelet monoamine oxidase (MAO) activity has similarly been studies as a measure of serotonergic function. Biederman et al (1984) reported reduced MAO activity in anorectic patients with depression, but normal in non-depressed anorectics and controls. This is, however, the only study to date.

Neuroendocrine studies

Few studies have been carried out in anorectic patients. Goodwin et al (1989) report no alteration in PRL responses to L-tryptophan in anorectics compared with controls, although Brewerton et al (1990) reported blunted PRL responses to both L-tryptophan and the 5-HT agonist MCPP in underweight anorectic patients compared with controls. Furthermore, responses to these challenges carried out four weeks after reaching goal weights were also found to be blunted. This however, could simply reflect their ongoing low body weights as ABW was only 85% ±3%, and long-term recovery studies would be needed to answer this question.

Blunted responses can also occur secondary to low prolactin stores or impairment of

pituitary lactotroph function. However, as PRL responses to TRH have been found to be largely (Kiriike et al, 1987; McBride et al, 1991), but not always (Waldhauser et al, 1984) normal in underweight anorectic patients, this explanation seems unlikely.

An alternative explanation, receiving much attention, is that 5-HT receptor responsivity of the pathways mediating the PRL responses to these challenge drugs are blunted in anorexia, regardless of patients' weights and nutritional status at time of testing (Brewerton et al, 1990). This theory is further supported by the finding of a trend towards blunted PRL responses to the 5-HT releasing drug dl-fenfluramine in a mixed group of acutely ill/partially refed patients. This did not show normalisation following six to ten weeks of apparently successful treatment (McBride et al, 1991).

These results are in contrast to those of Goodwin et al (1989) who failed to find blunted PRL responses to an L-tryptophan infusion in acutely ill patients compared to controls. However, this paper reports a significantly blunted GH response to the challenge, suggesting different receptor mechanisms for the hormonal responses. They suggest that this response may be related to reduced DA function secondary to dietary factors.

1.3.3.2 5-HT and Bulimia Nervosa

Precursor and metabolite studies. It has been argued that bulimic behaviour is consistent with hyposerotonergic activity (Kaye et al, 1991). Few studies of peripheral 5-HT function have been published. Lydiard et al (1988) report no baseline plasma tryptophan/LNAA ratio differences in normal weight patients compared with controls, suggesting normal tryptophan availability. However, a naturalistic but uncontrolled lab study of actively

bingeing patients reports an inverse relationship between the development of satiety (defined as cessation of bingeing and vomiting) and the ratio of plasma tryptophan to other LNAAs (Kaye et al, 1988b).

It is suggested that this increase in plasma tryptophan/LNAA ratio may be associated with the termination of a binge episode through its effects upon brain 5-HT metabolism. This suggestion is further supported by their finding that those patients who exhibited increased plasma tryptophan/LNAA ratios were also found to have greater increases in plasma PRL, suggesting that enhanced brain 5-HT neurotransmission had indeed occurred.

A number of studies have reported normal CSF 5-HIAA concentrations in normal weight bulimic patients (Jimerson et al, 1988; Kaye et al, 1990c). However, Jimerson et al (1988) also report that those patients with a binge frequency of at least twice daily were found to have lower CSF 5-HIAA levels than the less symptomatic patients and the controls. These findings did not appear to relate to differences in body weight or to a history of depression, and suggest that higher frequencies of bingeing may be 'associated with relatively persistently reduced central serotonin turnover' (Jimerson et al, 1990). However, whether these findings represent serotonergic dysregulation intrinsic to the development of the disorder, or are the consequences of persistent bingeing and purging behaviour and/or nutritional disturbances has still to be determined.

Platelet studies. To date, very little work has been published in this area. Increased platelet 5-HT uptake has been reported in actively purging and bingeing normal weight bulimic patients compared with controls (Goldbloom et al, 1990). As uptake did not correlate with depression, body weight, frequency of recent bingeing and vomiting

behaviour, it suggests that this finding reflects a decreased availability of 5-HT. The authors suggest that this may be at postsynaptic receptors at the hypothalamic level, thus reducing satiety signals, and possibly altering macronutrient choice towards increased carbohydrate preference (Goldbloom et al, 1990).

In a single preliminary report of platelet ^{3}H-imipramine binding, a group of non-depressed bulimic patients were found to have a significantly reduced density of binding sites compared to controls (Marazziti et al, 1988).

Neuroendocrine studies. Again, relatively few studies have been carried out, but those that have been reported provide evidence for serotonergic dysregulation. Peak PRL responses to mCPP are blunted in bulimic patients reaching DSM-III and DSM-IIIR criteria when compared with controls (Brewerton et al, 1992). This is not dependent upon the presence of major depression, although the PRL response to an L-tryptophan challenge in the same group of patients was found to be blunted only in those patients who concurrently met criteria for major depression.

The blunted PRL responses to mCPP were not related to age, body weight, medication, plasma drug levels or baseline oestradiol levels. Similarly, the possibility that they were due to alterations in pituitary lactotroph activity is not supported by work demonstrating that bulimic patients have normal PRL responses to a TRH challenge (McBride et al, 1991; Levy et al, 1988). However, an inverse correlation was found between PRL responses following the mCPP challenge and baseline cortisol levels in the bulimic patients. This latter finding parallels animal work where PRL responses to mCPP were found to be blunted following long-term cortisol treatment (Bagby et al, 1989), and raises the possibility

of an inverse relationship between 5-HT function and the hypothalamic-pituitary-adrenal axis similar to that debated for human studies in depression.

The different hormonal responses to the two serotonergic drugs could reflect a number of factors including differential involvement of presynaptic (L-tryptophan) and postsynaptic (mCPP) mechanisms, different 5-HT receptor subtypes mediating the responses of the drugs, or different anatomical loci of action (Brewerton et al, 1992).

Whilst careful controls for recent dieting, bingeing and purging behaviour (over four weeks prior to study) were made in this study, it is still not possible to conclude whether the blunted PRL responses reflect 5-HT dysregulation consequent to long-term bingeing and purging behaviour not yet normalised, or whether it reflects a 'premorbid state that predisposes these patients to bulimia nervosa' (Brewerton et al, 1992).

PRL response to dl-fenfluramine show a trend towards blunting in a group of eating disordered women (McBride et al, 1991), although only four of the group of seven met criteria for bulimia nervosa at the time of the study, making interpretation of this finding difficult.

In keeping with other 5-HT neuroendocrine challenge studies, Goldbloom et al (1990) offer evidence for reduced serotonergic activity in this patient population using 5-HTP as the challenge. They report that both GH and PRL responses to intravenous 5-HTP were blunted in patients compared with controls.

1.3.3.3 Current status of 5-HT research in eating disorders

Anorexia nervosa. In balance, research findings so far suggest that 5-HT dysregulation does occur in anorexia nervosa, although the precise nature of the dysregulation remains unclear. Whilst it is reasonably clear that the low CSF 5-HIAA levels seen in acutely ill patients probably reflect starvation, it is also possible that restrictor- and bulimic-type anorectic subgroups can be separated on the basis of central 5-HT turnover rates, the former having higher rates than the latter. The finding that CSF 5-HIAA levels are elevated in long-term recovered anorectics (Kaye et al, 1991) further raises the possibility of increased 5-HT neurotransmission in individuals with this disorder. Increased serotonergic activity is theoretically consistent with many features seen in anorexia nervosa and raises the intriguing possibility that anorectic patients may reduce food intake in order to lower brain 5-HT activity.

The blunted hormonal responses to mCPP, L-tryptophan and dl-fenfluramine may appear at first glance to contradict the CSF metabolite results, in that they suggest reduced 5-HT neurotransmission. However, all of the neuroendocrine studies published so far have been carried out on either acutely ill patients, or on patients who have only recently recovered, and in whom body weight often remains low and menstrual functioning impaired. As with the metabolite studies, effects of starvation, low body weight and/or partial refeeding could still be having a significant influence at this stage.

Bulimia nervosa. Evidence also exists from precursor and metabolite studies, platelet re-uptake findings, and three separate 5-HT neuroendocrine challenge probes to suggest that disturbances in 5-HT function do occur. It remains unclear whether these abnormalities

persist beyond the acute and early recovery phases of the disorder, and long-term recovery studies are still awaited. In balance, the evidence supports an explanation of hyposerotonergic activity, which is theoretically consistent with many of the features and behaviours associated with bulimia nervosa.

Research of clinical eating disorders needs to address the limitations of existing research strategies. An important consideration is that of determining when an individual patient's nutritional status and weight has been restored to a level at which normal functioning can be assumed (Goldbloom et al, 1991), thus removing the confounding factors of starvation and low body weight. Starvation is associated with significant cognitive, affective, and behavioural changes (Garfinkel et al, 1985), along with metabolic alterations (Sharp et al, 1993). While starvation effects have obvious consequences in anorectic patients, it is increasingly recognised that they probably play a major role in bulimia nervosa as well (Gwirtsman et al, 1989b).

In addition to weight changes and nutritional effects, behaviours such as bingeing and purging have biological consequences which must also be carefully controlled. These factors must be considered when trying to separate biological abnormalities that occur either as a consequence of the disorder or are important in symptom maintenance (eg starvation effects), from those that might predispose an individual to the development of eating difficulties. Studies of long-term recovered patients would be a fruitful way of assessing underlying brain 5-HT function of these populations. In addition, it would be beneficial to examine the confounding factors themselves (such as the effects of weight loss on brain 5-HT function) thus enabling a more accurate interpretation of the currently available research.

1.4 DIETING BEHAVIOUR

Dieting is a very common behaviour in western cultures, especially amongst women. There is a growing empirical link between dieting behaviour and the development of eating disorders (ie anorexia and bulimia nervosa). This topic will be reviewed in section 1.4.2. With the diminishing age of onset of eating disorders, the focus of concern has been on the adolescent population.

Little data exists to define rates of dieting in the general population, but several studies have concentrated on adolescent populations. Johnson et al (1983) report that 69% of a large cohort of adolescent girls (n=circa 1300) had dieted at some point, and that 37% were currently dieting. Abraham et al (1983) report figures for a group of 15-27 year old women (n=100) of whom 86% had dieted. Nylander (1971) compared all 14-19 year old males with females in one town (n=1129 vs 1241). This comparatively early study reported that 5% of 14 year olds had engaged in dieting behaviour, but that among 18 year odds, 44% of the females had dieted compared with 4% of the males. The data from these studies have been collected from American, Swedish and British populations.

1.4.1 DIETING AND PSYCHOLOGICAL FUNCTION

Food restriction clearly affects an organism's ability to function well. However, despite the importance of adequate nutrition, relatively little research has been carried out on the effects of dieting and food restriction in human populations. Much of the existing literature on dieting in normal populations has grown from the literature describing eating disorders, and has therefore often been a series of analogue studies seeking to understand clinical eating

disorders using populations of healthy volunteers. Thus, behavioural manipulations within the normal range are used as analogues for disordered behaviour.

More recently, however, emphasis has been placed on examining the effect of normal eating behaviour independently of eating disorders; the effects of dieting have been examined in relation to psychological functioning and patterns of eating behaviour in healthy subjects.

Effects of Dieting on Psychological Functioning

Very little work has been carried out in the area of how dieting may affect psychological functioning; the first direct evidence for the effects of food restriction on psychological functioning comes from the Minnesota study (Keys et al, 1950) which identified a large number of psychological changes as well as physiological changes associated with weight loss over 24 weeks. The main findings were that this regime resulted in an increase in lethargy, tiredness, depression, irritability, and a lowering of energy. These findings were not reflected in the more objective measures, although this is likely to be an artifact of poor study design.

Recently it has been shown that female dieters perform less well on demanding cognitive tasks (Rogers and Green, 1993) when compared with non-dieters of both high and low restraint. Restraint refers to "the tendency to control food intake consciously in order to prevent weight gain or promote weight loss" (Tuschl, 1990), and can be measured using standardised self-report measures such as Eating Attitudes Test (EAT). The finding of Rogers and Green (1993) has been replicated (Green et al, in press); here the range of tasks included in the study indicated that these deficits may encompass a range of behaviours

including reaction times and memory (impaired recall). Now further research is needed to determine more precisely the areas of cognitive functioning which are impaired. This has important implications for our understanding of the cognitive deficits that have been reported in eating disorders (see below).

Very little direct work has been carried out in the area of cognitive functioning in eating disorders; one group of researchers have reported impaired performance in a group of patients with bulimia nervosa compared with healthy controls (Laessle et al, 1990). Here there was a correlation with metabolic signs of starvation and size of impaired performance on the tasks. Fox (1981) reports a general impairment in restricting anorexics, and this has been replicated by Hamsher et al (1981) who characterise these impairments as an attentional deficit.

Presently it is not clear whether these effects are a result of food deprivation or an intrinsic aspect of the eating disorders.

Many of the neuropsychological studies carried out in normal populations have been retrospective and have divided subjects up into groups on the basis of reported behaviour rather than being prospective where subjects are assigned to a particular group. Pre-existing group differences may therefore have accounted for some of these findings.

The effects of dieting on mood

The issue of changes in mood associated with dieting/food restriction is controversial. Very few studies have examined the effects of dieting on mood in healthy populations. Keys et

al (1950) report a decrease in mood on a 24 week semi-starvation diet. This type of finding has been replicated by Fichter et al (1985) in five healthy volunteers placed on a three week starvation regimen. In contrast Pirke et al (1985) found no change in mood across a six week diet, and Warren and Cooper (1988) report no change across a two week dieting period.

It is not clear whether the presence of lowered mood and depression is caused be the food restriction, or is a component of the disorder itself. The issue of causality in dieting and lowering of mood therefore needs to be investigated in order to more fully understand the role of dieting in eating disorders.

1.4.2 DIETING, EATING DISTURBANCE AND EATING DISORDERS

A concern for the role of dieting behaviour in eating disturbance and eating disorders comes from several areas of research encompassing mainstream psychology and more directly, clinical studies of eating disordered populations.

Parenthetically, the literature in this area uses the term dieter and restrained eater interchangeably. It has been argued that dieting and restrained eating are not the same (eg Green, in press), and that the synonymy of the usage of these terms is fallacious. Dieting is a behaviour designed to bring about weight loss and therefore demands a relatively severe degree of food restriction. Dietary restraint however, has been defined as "...the tendency to control food intake consciously in order to prevent weight gain or promote weight loss." (Tuschl, 1990). Measures of restraint typically contain items reflecting concern with dieting

and weight fluctuation, with no attempt to measure current dieting behaviour. This study is concerned with dieting behaviour specifically, and I will therefore limit my review to studies of dieters where possible.

Psychology has contributed greatly to our understanding of the link between dieting and disordered eating. Seminal work carried out by Herman and Polivy in the 1970's (see Herman and Polivy, 1988 for review) has unequivocally demonstrated the link between restrained eating and disordered eating. They conducted a series of laboratory studies in which they demonstrated that dieters fail to compensate for a preload of food at a subsequent intake. That is, dieters did not compensate for a modest calorie loading by decreasing their caloric intake at next meal. The original study had been carried out in obese subjects by Schachter et al (1968). They found that obese subjects ate as many crackers ad lib immediately after filling themselves up on sandwiches (the preload condition) as they did when they received no sandwiches beforehand (no preload/ deprivation condition). In contrast, the normal weight subjects compensated for the sandwich preload, eating significantly less than in the no preload condition. Herman and Mack (1975) replicated this study using milkshake preloads but, importantly, substituting dieters for obese subjects. The dieters exhibited the same tendency of failure to compensate for the preload, but unlike the obese subjects, they actually ate **more** after the preload than after no preload; ie to 'counterregulated' rather than showing the normal compensatory regulation seen in the normal controls. This finding has subsequently been replicated many times (eg Polivy et al, 1986). It had been widely argued that the counterregulation effect is a result of the preload causing disinhibition in the dieter, with resolve to maintain the diet being eroded. Put simply, the dieter responds to the preload with such thoughts as 'I've blown my diet, I may as well continue to eat'. This cognitive explanation gains further

support from the finding that dieters also counterregulate when they **perceive** the preload as high in calories regardless of the actual calorie content (Spencer and Fremouw, 1979). However, there is little direct evidence that this cognitive process is necessary to the process of counterregulation which occurs in dieters, or that it is indeed present (Jansen 1990; Jansen et al, 1988).

This type of psychological modelling was originally developed in order to better understand the mechanisms involved in obesity, but as noted the dieters actually respond differently to the obese subjects in these experiments. However, it has better been applied to theories of binge eating. Binge eating is a common behaviour in dieting, and these lab demonstrations of counterregulation can be regarded as on a continuum with more severe episodes of binge eating which occur in bulimia nervosa. It has proven very difficult to make the comparison between dieters and bulimics scientifically because of the secret nature of the disorder, and the difficulties of producing true binges in a lab setting. Thus essentially an experimental view of dieting is being compared with an almost exclusively clinical view of bulimia.

Correlational studies have added further support to the theory that dieting and bingeing are causally connected. There is considerable evidence that these two behaviours co-occur; a sub-group of patients with anorexia nervosa experience episodes of binge eating in the context of severe dietary restriction (Beaumont et al, 1976). This subgroup have been shown to score higher levels of restraint than nonbulimic anorexics ((Herman and Polivy, 1980). Normal weight bingers tend to describe themselves as dieters (Pyle et al, 1981), and college students with high restraint scores also report significantly more episodes of binge eating compared with low scorers (Hawkins and Clement, 1980).

Correlational data cannot directly contribute to the theory that dieting **causes** binge eating; it could equally well be argued that the binge eating causes dieting in that individuals would wish to compensate for their excessive eating, and would therefore subsequently restrict their food intake. Lab studies as described above, again, do not offer direct evidence of the natural order in which these behaviours would occur outside the lab. Evidence for the causality of dieting in binge eating comes from animal studies and from data on clinical populations.

Young female rats will gain weight on an ad libitum diet of palatable food. However, a group of previously food deprived rats gained significantly more weight than their controls, and this gain was in direct proportion to the length of their prior deprivation (Coscina and Dixon, 1983). Importantly, this differential weight gain was shown to be a result of differences in eating rather that to differences in metabolism. This animal model suggests that prior deprivation influences tendency to overeat even after weight loss has been restored.

With regard to human populations, clinical reports on bulimic anorexic patients offer further support that dieting may lead to overeating, or in this case, binge eating. Clinical case reports indicate that these patients very often have marked periods of weight loss in the calendar year preceding the onset of bulimia (Russell, 1979). Garfinkel et al (1980) demonstrated that in a group of 68 bulimic anorexic patients, their bulimia developed a mean of 19.2 ±8 months after the onset of dieting. This pattern has been replicated in cases of non-anorexic bulimia, being true in more than 90% of cases. Further causal evidence comes from studies of obese populations. Telch and Agras (1993) demonstrated that 62% of obese non-binge eaters reported binge eating episodes in the 3 month period immediately

after a 12 week very low calorie diet. Whilst these episodes did tail off, it is easy to imagine that if dieting behaviour persisted, there would be an increase in binge eating, culminating in a cycle of bingeing and dieting. However, there was no correlation here between weight loss and binge status at follow-up, and no other study has attempted to pursue the question of whether weight loss per se, or a specific degree of weight loss is associated with a risk of binge eating.

Whilst clinical studies have provided valuable data, there is very limited data from studies of normal populations. The most dramatic evidence comes from the Keys et al (1950) study of World War II conscientious objectors, who were placed on a 24 week semi-starvation diet resulting in 26% weight loss. Upon refeeding, these men returned to their normal weights but exhibited a persistent tendency to binge, to the extent of gorging at meals to the limit of their physical capacity. This type of behaviour had not existed in these men prior to the study. Warren and Cooper (1988) placed 14 people on a three week calorie-controlled diet, They found an increase in the ratings of preoccupation with thoughts of food, urges to eat more frequently, and feelings of being out of control of their eating. These changes in cognitions concerning eating are those often associated with binge eating. This study however, did not attempt to explore the dieters behaviour after cessation of dieting, and it is therefore difficult to compare these findings with the models outlined above.

Further evidence for the role of dieting behaviour in the development of eating disorders comes from a small number of studies which have adopted a prospective design in 'at risk' groups. Patton (1988) followed up a cohort of 1010 schoolgirls over a 12 month period in order to assess which factors predicted outcome. On the basis of the initial screening

and interview, subjects were divided into one of three groups (dieters, non-dieter, case). Follow-up on a smaller cohort indicated that the most change in eating behaviour occurred in the diet group. Of these 42% were re-classified as non-dieters, but 21% had become cases, and represented half of the case population. This was in contrast to the original non-dieter group of whom only 0.03% had been reclassified as a case. This study, then, identifies dieting as an important aetiological factor in the development of eating disorders. This has recently been replicated in a similar study where it is reported that the risk of dieters becoming cases is eight times that of non-dieters (Patton et al, 1990).

In conclusion, it can be seen that there is evidence from a wide range of studies suggesting that dieting is an important risk factor in the development of eating disorders. The mechanisms which may be important in the transition of normal dieting behaviour to abnormal eating disordered behaviour are, however, poorly understood. It would be important to pursue theoretical explanations of what these mechanisms may be. One interesting area centres on the effects of nutrient intake and neurobiological function. This work will be reviewed in the following section.

1.4.3 NEUROBIOLOGICAL EFFECTS OF DIETING

It is important to understand the cognitive effects of dieting, but these are mediated by neurobiological changes which occur in response to the dieting behaviour itself. The effects of dieting and food deprivation have therefore been examined in relation to neurobiological function. This type of research is important in our understanding of the effects of altered nutrition on brain function, and hence behaviour. This in turn contributes to our understanding of psychiatric illness. With regard to eating disorders, a second important

contribution is that of disentangling which neurobiological abnormalities are a result of food deprivation, and which are specific to the aetiology of the disease itself.

The present thesis focuses on 5-HT which has an important role in the control of appetite and mood (see section 1.5.2 and 1.1.1). Evidence suggests that dieting alters the function of this neurotransmitter, and these findings may have implications for the development of eating disorders (section 1.4). Thus, the following review will focus on studies carried out in relation to 5-HT function. The effects of food intake and dietary restriction on levels of neurotransmitter precursors, and on the synthesis of brain neurotransmitters will be examined. With regard to human functioning, studies examining the neurochemical effects of dieting will be included.

Amino acid precursor supply in relation to dietary restriction

It has often been argued that mechanisms have evolved which regulate our food intake to ensure that we maintain adequate supplies of amino acids, and thus that protein intake is at least partly under direct control (eg Hill and Blundell, 1988). It has further been argued that subsequent changes in plasma and brain profiles of amino acids may regulate food intake and appetite (Hill and Blundell, 1988).

Most research has centred on the amino acids tyrosine and tryptophan, although studies of the effects of fasting have encompassed most amino acids. Short periods of fasting (24-120 hours) have been shown to have little effect on plasma concentrations of most amino acids in the rat (Enwonwu, 1987). In this study, levels of plasma phenylalanine increased, whereas alanine, arginine, glutamate and threonine showed a moderate decrease. Brain

concentrations of amino acids remain largely unaltered, although their levels are determined by plasma concentrations (Knott et al, 1973), such as increase in phenylalanine, and decreases in alanine and arginine. However, for two amino acids the impact of fasting on brain values is not reflected in changes in plasma concentrations; plasma threonine falls significantly with 24 hour fasting (Enwonwu, 1987), but remains unaltered in the brain. Conversely, it has been argued that concentrations of total plasma tryptophan remain unaltered in response to fasting, but levels in the brain are increased (Curzon et al, 1972). It has also been noted that brain tryptophan increases if rats are given a large carbohydrate meal which is tryptophan free (eg Fernstrom and Wurtman, 1971a).

A preliminary explanation of these paradoxical findings on brain tryptophan has been put forward by Curzon (1988); here it has been argued that fasting increases plasma unesterified fatty acids which in turn displace tryptophan from albumin to increase its availability for transportation to the brain (Knott and Curzon, 1972; Curzon et al, 1973). The effects of a high carbohydrate meal would cause insulin release, and a resultant increase uptake of large neutral amino acids (LNAA's) into muscle. This would then lower the ratio of LNAA's to tryptophan, thus enhancing transportation of tryptophan to the brain (see paragraph 1.5.5 for an elaboration of this process).

It can be seen therefore that the relationship between intake of tryptophan, plasma levels and brain concentrations is complex after short term fasting in the rat. It is unclear how well these findings may relate to human functioning, especially to sustained food restriction as opposed to total fasting. Schweiger et al (1986) examined this issue in a healthy population, and reported that there were no differences in the ratio of tryptophan to other LNAA's over a six week diet compared with controls. However, there was no indication

of what the absolute levels of plasma tryptophan were, and it is therefore difficult to put this finding in context, especially in the light of the theory of Curzon (1988).

Effects of dietary restriction on brain 5-HT function

A second approach to this area has been to indirectly examine the functional status of brain transmitter function, and this has been carried out using a neuroendocrine challenge paradigm in relation to brain 5-HT function in humans.

A small number of studies have been carried out to examine the effects of moderate dieting in healthy human volunteers. The effects of a 3 week, 1000 kcal diet on neuroendocrine responses to L-tryptophan (LTP) suggests that dieting is associated with an increased prolactin response to the challenge (Anderson et al, 1990b, Goodwin et al, 1987). Importantly, this effect was seen in women only. This study also demonstrated that dieting is associated with a decrease in total plasma tryptophan and a decrease in the ratio of plasma tryptophan to branch chain amino acids (BCAA's) in both men and women. These findings suggest that dieting results in a decreased availability of plasma tryptophan for brain 5-HT synthesis in both men and women, but than only in women was this associated with a functional alteration of brain 5-HT status as measured by the prolactin response to LTP. Further work has been carried out in order to understand the mechanisms involved in these diet induced changes in brain 5-HT function. Research has been carried out using the selective pre-synaptic 5-HT agonist, d-fenfluramine. Prolactin response to d-fenfluramine were significantly enhanced after dieting in women but not men (Walsh et al, 1993), giving findings consistent with the previous studies.

It has been argued that the prolactin response to LTP may not be 5-HT mediated. However, further research has examined the effects of metoclopramide, a dopaminergic antagonist in this paradigm; here there was no effect on the prolactin response to metoclopramide in dieting volunteers (Anderson et al, 1989), thus suggesting that dieting selectively alters 5-HT mediated prolactin release.

From the above studies, it is clear that in healthy volunteers, dieting has marked effects on levels of plasma tryptophan, and in women this appears to have functional significance for brain 5-HT function. It is unclear what the importance of these effects on psychological functioning may be; neurobiological theories of eating disorders suggest that alterations in brain 5-HT neurotransmitter are associated with the pathology of the disorder (section 1.3.5), and importantly, dieting behaviour is a risk factor in the development of eating disorders (section 1.4.2). Furthermore, given the marked preponderance of eating disorders among women (Anderson et al, 1990b) the findings of the above studies are of particular relevance to theoretical investigations of underlying mechanisms; the finding that women are more vulnerable to the effects of dieting may shed light on why dieting behaviour may precede eating disorders, and why the prevalence rate is so high in the female population. These theoretical implications suggest that it would be important to examine further the effects of dieting on neuropsychological functioning and to examine whether there are correlations between changes in these measures and alterations in plasma tryptophan. Work carried out for this thesis addresses some of these issues.

1.5 APPETITE

1.5.1 A DEFINITION OF APPETITE

In order to successfully examine the effects of pharmacological manipulations on human eating behaviour, it is necessary to have a conception of the set of behaviours and physiological drives which work to maintain an adequate supply of food for the individual. There is clearly a complex relationship between eating behaviour and metabolism, and thus it is necessary to break down these processes into discrete units.

Both in human and animal studies, researchers have traditionally adopted a quantitative approach to appetite, and regarded a simple measurement of food intake within a discrete period of time, and with a diet of fixed nutritional composition as an adequate measure of this (Tedeschi, 1966). However, this very restricted conceptualisation of appetite is inadequate as a means of assessing the true impact of pharmacological agents. Models developed more recently in animal research examine the microstructure of feeding behaviour, where an analysis of appetite includes a wider range of feeding related behaviours (such as rate of eating, number of meals, meal size and duration) and an appreciation of the importance of assessing these behaviours over time (Blundell, 1984). Fig 1.4 depicts this conceptualisation of 'the satiety cascade', a model which incorporates the factors contributing to satiety. In addition to this, researchers have realised the importance of providing the animal with a range of macronutrients in order to observe effects of neurotransmitter manipulations on macronutrient intake (Ashley et al, 1979).

A theoretical framework which includes the qualitative and quantitative aspects of appetite

related behaviours in man has been put forward by Blundell (1988). This model incorporates a consideration of temporal changes in eating, sensory, cognitive, post-ingestive and post-absorptive aspects of food intake (Fig 1.4). Methodological limitations make it very difficult to examine eating behaviour over long periods of time in human populations, but this model highlights the necessity of measuring cognitive parameters. In addition, it can be seen that measurement over time enables an inclusion of the effects of post-ingestive and post-absorptive mechanisms. This model, then, considers the elements involved in eating behaviour along with the various processes which can be regarded as mediating the cascade of events leading to satiety.

Working within this framework demands a precise definition of terminology. In a study of appetite, it is necessary to separate the parameters which dictate an 'urge to begin eating' with those that are concerned with 'the amount consumed'. Hunger, then, can be regarded as the process with stimulates the onset of eating, and satiety refers to the state which inhibits further eating (Blundell, 1984). Clearly, these measures rely on self report of subjective state, and are insufficient in themselves to provide a clear picture of human appetite. Objective measurement of actual food intake, and it's constituents allows an accurate assessment of changes in energy consumption. Furthermore, it is possible to calculate intake of macronutrients and other nutrients in a similar way. With regard to the studies carried out in this thesis, a consideration of macronutrient consumption is directly related to the hypotheses under investigation. These are defined as the proportion of food made up of carbohydrate, protein and fat.

Fig 1.4



Conceptualization of the satiety cascade indicating the likely contributions of sensory, cognitive, post-ingestive, and post-absorptive processes to the intensity and time course of satiety (From Blundell, 1988).

1.5.2 THEORIES OF APPETITE

Appetite research encompasses several approaches, the most important of which are reviewed below. Classical neuroanatomical studies have done much to elucidate the brain regions important in the control of food intake, and studies of neurotransmitter function haveprovided insight into how these mechanisms may operate in order to regulate appetite and maintain adequate supply of nutrients. This approach offers the opportunity to examine the abnormal functioning of appetite and some of the determinants of any abnormality.

1.5.2.1 Neuroanatomical Theories of Appetite

Early studies identified that damage to the base of the brain in or close to the hypothalamus led to overeating and obesity in clinical cases (Rolls, 1981). Subsequent research identified the ventromedial hypothalamus (VMH) as being the critical region; bilateral lesions here result in hyperphagia and obesity (Grossman, 1973). Electrical stimulation to this area causes cessation of eating (Rolls, 1981). Lesions in the lateral hypothalamus (LH) have been identified as causing aphagia (Rolls, 1981), suggesting that both centres are important in controlling food intake. These studies suggested that these hypothalamic centres acted together with the LH as a feeding centre, and the VMH as a satiety centre (Stellar, 1954; Grossman, 1973).

Criticisms of this work centre on the problems of non-specificity of the lesions; damage to areas outside the LH can also produce aphagia (Marshall et al, 1974). Moreover, this type of damage was associated with sensorimotor dysfunction causing problems with orientation. However, more recent studies using neurophysiological techniques of recording single cell

activity of single neurones in primates, suggests that populations of cells in the hypothalamus are important in the control of feeding behaviour (Rolls, 1981). This is, however, a limited conceptualisation of the role of higher CNS mechanisms; more recently the orbitofrontal cortex in primates has been associated with information of sight, smell, taste, and texture of food (Critchley, 1993). This centre is part of a circuit comprising the amygdala, LH, and striatum, all shown to be important in the autonomic feeding response (Rolls (1993).

1.5.2.2 Neurobiological Theories of Appetite

Evidence from animal and human studies suggests that food intake is mediated in part by specific neurotransmitters, and that these are responsive to the modulatory effects of circulating nutrients and hormones (Leibowitz, 1987). 5-HT is known to have an important role in the regulation of feeding behaviour, and this will be reviewed separately in section 1.5.2.4.

Central feeding system

Noradrenaline (NA). Microinjection studies reveal that direct application of directly or indirectly acting alpha-two agonists such as clonidine to the paraventricular nucleus (PVN) causes pronounced hyperphagia. This can be modulated by lesioning of the afferent pathways (Leibowitz and Brown, 1980). Injection of NA increases meal size rather than meal frequency (Shor-Posner et al, 1985), and increases carbohydrate consumption (Leibowitz, 1985).

The mechanisms underlying the anorectic effect of amphetamine have been explored, and evidence suggests that whilst the euphoriant effect of amphetamine may be mediated via DA release, the anorectic effect is mediated by NA (Silverstone et al, 1980, Blundell and Latham, 1980). It remains unclear, however, whether this effect is mediated by α- or β-receptors. The non-specific β-adrenergic antagonist, popranolol, completely attenuated d-amphetamine induced anorexia in rats (Sanghvi et al, 1975) but not mice (Dobranzanski and Doggett, 1979). The β-adrenergic agonist, salbutamol, is associated with reduced food intake in rats, but this effect is prevented by pretreatment with popranolol (Borsini et al, 1982). In contrast, the α-blocker, phentolamine, augments amphetamine-induced anorexia in rats and mice (Sanghvi et al, 1975; Dobrazanski and Doggett, 1979). These studies suggest that the role of NA in amphetamine-induced anorexia is via the β-adrenergic receptor.

Dopamine (DA). This neurotransmitter was originally thought to have an important role in appetite regulation (Morley and Blundell, 1988). However, the effects of 6-hydroxydopamine administration may alter food intake secondary to other behaviours, and that depletion of DA results in low levels of arousal and impaired sensorimotor integration which in turn affect feeding behaviour (Morley and Blundell, 1988). Furthermore, the effects of DA appear to be dose dependent with large doses of DA and DA agonists having an inhibitory effect on eating, whereas lower doses stimulate feeding (Essatara et al, 1984). The specific role of DA remains to be determined, although studies have also examined the role of DA in the mediation of hedonic responses to food. The effects of neuroleptic administration on the consumption of sweet, palatable foods have been examined (Xenakis and Sclafani, 1981). Demonstrations of increased consumption of these foods suggest that DA may mediate feeding behaviour by a process of positive effects associated with

consumption of these food types (Morley and Blundell, 1988).

Finally, with regard to the role of DA in the control of appetite in humans, evidence comes from clinical reports of the well known association between obesity and weight gain in patient populations maintained on major tranquilizers such as the butyrophenones (eg haloperidol and trifluperidol), and phenothiazines (eg chlorpromazine, methotrimeprazine).

Opioids. Opioid antagonism has been demonstrated to decrease feeding in many species, both mammalian and non-mammalian (Kavaliers et al, 1984, Morley et al, 1983). However, the efficacy of opioid antagonism is determined by many variables including age of animal, and state of food deprivation, suggesting that the role of the opioids is not a central one (Morley and Blundell, 1988).

Opioids appear to modulate feeding via a number of different sites; the paraventricular nucleus (PVN) is regarded as the major site of action for dynorphin (one of the endogenous kappa ligands) (Gosnell et al, 1986), although the globus pallidus and the amygdala are also sensitive to the effects of opioids and related drugs (Stanley et al, 1984; Gosnell et al, 1984).

These effects have generally been confirmed in human studies. Naloxone decreases food intake in obese and lean humans (Atkinson, 1982; Trenchard and Silverstone, 1983). The effects of opioid antagonism appear to act preferentially to alter fat intake (Gosnell et al, 1986). Long-term administration of opiate antagonists appears to have only a minor effect on weight loss (Mitchell et al, 1984), and opiate addicts on normal calorie intake have lower rather than higher body weights suggesting that the chronic action of the drug differs

to the effects seen with acute administration. This has been explained by alterations in energy utilization (Morley and Blundell, 1988).

Neuropeptite Y (NPY).

This pancreatic polypeptide is associated with increased feeding in rats. Central injections of NPY to the third ventricle cause a pronounced increase in food intake (Clark et al, 1984). Similarly, injections into the lateral ventricle increase feeding and drinking irrespective of stage of feeding cycle (Levine and Morley, 1984). Furthermore, this increase is highly specific to carbohydrate foods (Stanley et al, 1985). The role of NPY in human feeding behaviour remains to be confirmed, although it has been suggested that weight gain associated with tricyclic antidepressant medication may be mediated by the effects of these drugs on NPY (Morley and Blundell, 1988).

The above studies have led to the need for an integrative theory of appetite. Leibowitz (1988) has proposed that appetite is regulated very closely by the hypothalamus. She argues that neurotransmitters within the hypothalamus monitor and integrate the complex sensory and metabolic input regarding the nutritional status of the organism, and that this system further regulates eating behaviour in response to that information. This theory suggests that peripheral endocrine and autonomic systems provide neural, metabolic, and hormonal information necessary for the homeostatic control of intake of energy and nutrition by the hypothalamus.

This theory is largely supported by the above studies. In many cases, depending upon the neurotransmitter or drug injected, the hypothalamus is found to respond selectively to alter

food intake, where other brain regions do not, or do so with much less potency (Leibowitz, 1988). A review of these studies enables the location of specific regions within the hypothalamus; the PVN, ventromedial nucleus, and the lateral portion of the hypothalamus are regarded as the most important centres within this system (Leibowitz, 1988). Eating behaviour can be stimulated by injection to the PVN of NA, Neuropeptide Y, Galanin, gamma-aminobutyric acid (GABA), opiates and corticosterone. Conversely 5-HT and cholecystokinin (CCK) exert an inhibitory effect on food intake when injected to the PVN, as do dopamine, NA, and GABA when injected to the perifornical region of the lateral hypothalamus (see Leibowitz, 1988 for review). Analysis of these effects in terms of macronutrient intake reveals that this is most often characterised by alterations in carbohydrate consumption, although the opiates and dopamine appear to alter protein and fat intake specifically. Thus, it seems that the hypothalamus does indeed have a major role in appetite regulation. However, studies have reported effects in other brain regions, (for example, the amygdala is sensitive to opiates), suggesting that the system is complex, with the possibility that the different centres cited may regulate specific aspects of appetite.

Peripheral feeding system

A number of gastrointestinal peptides have been implicated in appetite regulation. These include cholecystokinin (CCK), pancreatic glucagon, and bombesin-like peptides, which are released by ingestion of food, and are regarded as being signals for satiety.

Cholecystokinin (CCK).

CCK inhibits food intake in many species (see Smith et al, 1981 for review). Furthermore,

administration of highly selective CCK receptor antagonists increases intake in several animal species including rats (Weller et al, 1990) and pigs (Ebenezer et al, 1990). These findings have been replicated in humans; CCK reduces intake in normal weight and obese volunteers (see Gibbs and Smith, 1992 for review). It thus is clear that endogenous CCK is important in the control of food intake, although the precise mechanisms remain unclear. For example, it is not yet known whether blockade of CCK receptors type A and/or B are necessary to increase food intake (Gibbs and Smith, 1992), although recent studies suggest that CCK_A receptors mediate the action of the peptide (Moran, 1993).

Glucagon.

Administration of pancreatic glucagon inhibits food intake in many species (Geary, 1990). The effects of this are similar to that of CCK, ie dose related, rapid, and transient. However, the effects of glucagon appear to be sensitive to environmental conditions such as state of food deprivation (Weick and Ritter, 1986). It has been hypothesised that glucagon acts to inhibit food intake via the liver and the hepatic branch of the vagus on the basis of studies of selective hepatic vagotomy and selective administration via the hepatic portal vein (Geary, 1993).

Bombesin-like peptides.

Similarly to CCK and glucagon, there is robust evidence that bombesin reduces food intake in a range of species is robust (see Gibbs and Smith, 1992 for review). This effect also appears to be demonstrable in humans (Muurahainen et al, 1983), although very few studies have been carried out to date.

Structurally related peptides have also been shown to reduce food intake. For example gastrin-releasing peptide (Figlewicz et al, 1985) and neuromedin B, although this is less potent (Gibbs and Smith, 1988). Bombesin not only decreases meal size, but produces a dose-related extension of the intermeal interval (Mindell et al, 1985).

1.5.2.3 5-HT and Appetite

The role of 5-HT in appetite has been the focus of research for over 20 years; it has long been recognised that patterns of food consumption can be manipulated with pharmacological agents acting on 5-HT (Blundell and Hill, 1987a). A consistent phenomenon is that direct or indirect activation of 5-HT receptors results in a suppression of food intake (see Blundell, 1984; Garattini et al, 1989 for reviews). Conversely, reductions of 5-HT function have been reported as increasing food intake (Leibowitz, 1986; Blundell, 1984). With regard to human appetite three main approaches have been employed in the investigation of the inhibitory effect of 5-HT on food intake. These are: an examination of the effects of 5-HT precursors, the effects of direct and indirect 5-HT agonists, and the interaction of post synaptic receptor blocking drugs with those known to produce an anorexic effect. Animal and human findings will be reviewed below.

5-HT precursors and food intake.

Several studies have reported reduction in food intake in rats associated with administration of 5-HTP (Singer et al, 1971; Blundell and Leshem, 1975). This is true for both l-5-HTP and dl-5-HTP. Evidence suggests that these effects are mediated centrally, and that the alterations in the micro-structure of eating are similar to those seen with fenfluramine (ie

a reduction in meal size, with no change in meal number) (Blundell, 1984).

Administration of tryptophan is also associated with a dose-dependent decrease in food consumption (eg Morris et al, 1987; Gibbons et al, 1981), although there have been some negative studies (eg Peters et al, 1984). It has been argued, however, that these negative findings may be a result of insensitivity in methodology in examining a relatively subtle effect (Blundell, 1984). Blundell argues that it is necessary to monitor feeding over a four hour period in order to elicit the anorexic effect of tryptophan. Under these conditions, it can be seen that tryptophan reduces meal size and extends the post-meal interval (Blundell, 1984). Tryptophan is also associated with decreased food intake in human populations (Hrboticky et al, 1985; Silverstone and Goodall, 1984), although low dose studies (ie less than 0.1g) report no change (Silverstone and Goodall, 1986).

5-HT lesion and depletion studies of appetite

Bilateral injection of p-chlorophenylalanine (PCPA) to the cerebral ventricles of rats elicits marked hyperphagia and increase in body weight (Breisch et al, 1976). Knifecut lesions to the midbrain tegmentum and regions of the median forebrain bundle are associated with hyperphagia (Grossman et al, 1977; McDermott et al, 1977). Lesioning here is associated with depletion of NA as well as of 5-HT but correlational analysis reveals that the hyperphagia is significantly associated with forebrain depletion of 5-HT. Bilateral micro-infusion of 5,7-dihydroxytryptamine (5,7-DHT) into the ventro-lateral hypothalamus resulted in hyperphagia only when rats had access to a palatable diet which was high in fat (Waldbilling et al, 1981). It has been argued that this may account for negative findings

in this area (Blundell, 1984), where animals are often maintained on a chow diet low in palatability. Blundell argues that this regimen may best suit studies of inhibition of eating, but act against eliciting an enhancement of intake.

Studies in humans have largely supported the theory that decreased 5-HT function is associated with an increase of eating behaviour. Cyproheptadine, an antihistamine compound and 5-HT receptor blocker, induces weight gain with chronic administration (Lavenstein et al, 1962) in asthmatic children. Furthermore, controlled studies report weight gain and increased hunger ratings over a three month period compared with placebo (Silverstone and Schuyler, 1975). Another 5-HT receptor blocker, methysergide, is also associated with weight gain and increased ratings of hunger (Silverstone, 1986; Graham, 1967). Goodall and Silverstone (1988) have demonstrated that the 5-HT receptor blocker, metergoline, attenuates the anorectic effects of d-fenfluramine on non-sweet foods, and was associated with an increase in sweet foods after four hours in healthy male volunteers.

Enhancement of central 5-HT and appetite

Animal studies. Central injection of small amounts of 5-HT to the PVN and perifornical area produces inhibition of eating, as seen with other catecholamines (see Leibowitz and Shor-Posner, 1986 for review). This is characterised by a decrease in meal size, duration and rate of eating. 5-HT and 5-HTP also inhibit NA-induced feeding by 90% when injected into the PVN (Blundell, 1984). D-Fenfluramine, which is more specific for the 5-HT system than its racemic form, produces marked anorexia (see Garattini et al, 1989 for review). The active metabolite D-norfenfluramine has even greater anorexic properties than D-fenfluramine (Garattini et al, 1979). Selective 5-HT reuptake inhibitors have also been

demonstrated as having anorectic properties; these include fluoxetine, zimelidine (Samanin, 1983; Blundell, 1984; Garattini et al, 1986), indalpine (Angel et al, 1988), although some studies report negative findings (eg Samanin et al, 1980).

These findings are seen in human studies of appetite; for example, fenfluramine suppresses appetite in healthy volunteers and has been used as an anti-obesity drug (see Silverstone and Goodall, 1986 for review). There appears to be a sex difference in response to dl-fenfluramine at higher doses (80mg) where Visual Analogue Scale (VAS) ratings of hunger are lower in females compared with actual intake at test meal which is increased compared with placebo (Silverstone et al, 1975).

5-HT receptor subtypes and appetite

The critical receptor subtypes for 5-HT mediated hypophagia have not yet been clearly identified. Preliminary studies have suggested that 5-HT_{1A}, 5-HT_2, and possibly 5-HT_3 are important in mediating this response.

5-HT_{1A} Selective 5-HT_{1A} agonists are associated with increased feeding in rats. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), ipsapirone, buspirone and gepirone all increase food intake in rats (see Dourish, 1986 for review). 8-OH-DPAT also increases feeding in mice, cats, pigs and rhesus monkeys (Dourish, 1986 for review). These compounds are thought to elicit this effect by their agonist properties at the 5-HT autoreceptor sites on cell bodies, which in turn, results in decreased 5-HT release in terminal fields (Kennett et al, 1987). Furthermore, 8-OH-DPAT induced feeding can be blocked by antagonists with high affinity for 5-HT_{1A} sites. These include the 5-HT_1/5-HT_2 antagonist metergoline and the

5-HT_{1A}/5-HT_{1B} antagonist (-) pindolol (Hutson et al, 1988).

5-HT_2 In food deprived and free feeding rats, quipazine, a 5-HT_1/5-HT_2 agonist, decreases food intake (Sammanin et al, 1977; Dourish et al, 1986). This effect is blocked by 5-HT_{2C}/5-HT_2 antagonists such as ritanserin and ketanserin (Dourish, 1985). MCPP and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) which both have high affinity for 5-HT_{2C} sites, are associated with decreased food intake in food deprived rats (Kennett and Curzon, 1988). Similarly, administration of the direct 5-HT agonist, MCPP, is associated with decreased food intake in the rat (Kennet and Curzon, 1991). MCPP binds most strongly to 5-HT_{2C} receptors, although it also has affinities for 5-HT_{1A} and 5-HT_{1B}. This group have demonstrated that the reduction in food intake is via activation of the 5-HT_{2C} receptors, although this mechanism depends on the presence of 5-HT_{1B} sites in the rat (Kennet and Curzon, 1988).

5-HT_3 The effects of compounds acting on these sites are poorly understood, and only a limited number of studies have been carried out. Selective 5-HT_3 antagonists, MDL 72222 and tropisetron, do not alter food intake in free feeding rats (see Dourish, 1986 for review). Large doses of the antagonist, ondansetron, increase the duration of feeding in mice (Shepherd and Rodgers, 1990). More recently, it has been reported that ondansetron and tropisetron are associated with a decrease in time spent feeding in rats (Van der Hoek and Cooper, 1990; Fletcher and Davies 1990).

Two studies have examined the effects of 5-HT_3 agonists on food intake; 1,3,-phenylbiguanide (PBG) and 2-methyl-5-HT decrease food intake when given i.p. (Simansky et al, 1991; Kennett and Grewal, 1992).

5-HT and macronutrient selection

5-HT has been suggested to play a role in the selection of macronutrients, that is, carbohydrate, protein and fat. There are two main areas of evidence which support this position. Firstly, 5-HT-enhancing drugs alter the pattern of macronutrients consumed, and secondly, it has been postulated that brain concentrations of 5-HT depend upon the nutrient composition of food consumed, and thus, that there exists a mechanism which determines selection of the correct proportions of macronutrients.

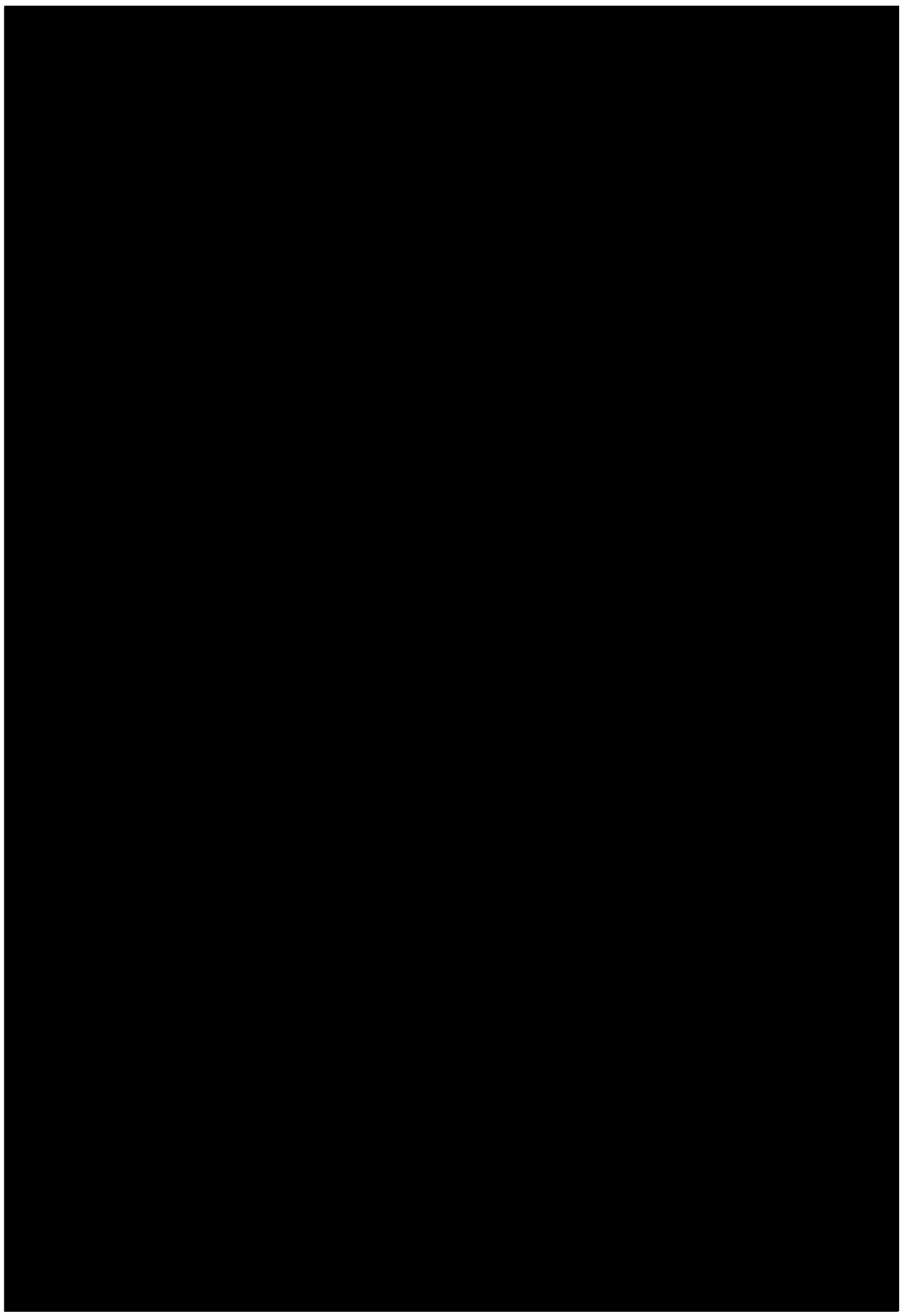
5-HT enhancement and macronutrients. Fenfluramine and fluoxetine have been shown to suppress food intake in food deprived rats. However, where experimentation allows for an analysis of macronutrient selection, it can be seen that animals select high protein food such that protein consumption is spared (Wurtman and Wurtman, 1977). In comparison, amphetamine which has little action at 5-HT sites, has been shown to equally diminish the consumption of macronutrients (Blundell, 1984). However, subsequent studies have revealed that this finding is not as clear cut in non-food deprived animals (see Blundell, 1984 for review). Here it is suggested that 'the role of 5-HT may be to control the proportion of carbohydrate to protein consumed rather than absolute amounts'.

The influential theory of Fernstrom and Wurtman (1972) describes how dietary influences alter the synthesis of brain 5-HT. The basic premise of this theory is that the amount of carbohydrate or protein in a meal will determine the ratio of tryptophan to other LNAA's, and this in turn determines the amount of tryptophan crossing the blood-brain barrier, and therefore the amount of tryptophan available for synthesis of brain 5-HT (see section 1.1.2 for review). This theory, then, regards the competitive transport mechanism as central to

the effects of food ingestion, of brain tryptophan and 5-HT synthesis. Food intake studies have demonstrated that the ingestion of a carbohydrate meal by fasting rats results in a rapid elevation of brain tryptophan levels, and a concomitant increase in the synthesis of brain 5-HT (Fernstrom and Wurtman, 1972). This is a result of increased insulin secretion which raises plasma tryptophan levels, and reduces levels of other LNAA's (Fernstrom and Wurtman, 1971b). This in turn gives tryptophan a competitive advantage for uptake into the brain. The ingestion of a protein-rich meal will also induce insulin secretion, raise plasma tryptophan, and lower levels of other LNAA's. However, the ingested protein will also provide an additional supply of amino acids, and thus the overall level of LNAA's will be elevated (Fernstrom and Fernstrom, 1988). Studies have shown that these elevations in tryptophan and LNAA's are comparable, and thus the ratio remains unchanged, and there is no change in brain tryptophan uptake or synthesis (Fernstrom and Wurtman, 1972; Fernstrom, 1983). This model is depicted in Fig 1.5.

This model has been used to describe eating disorders such as carbohydrate craving (Wurtman and Wurtman, 1986), and binge eating in bulimia nervosa (Kaye et al, 1988b). Here it is suggested that the alterations in eating behaviour (often characterised by consumption of large amounts of carbohydrate) are an attempt to alter the plasma ratio, and hence boost levels of brain 5-HT. Whilst the model remains controversial, it serves to illustrate how dietary influences may alter central neurotransmitter function.

Fig 1.5



Model describing diet-induced changes in the serum TRP/LNAA ratio, brain tryptophan levels, and brain serotonin synthesis. (T=tyrosine, P=phenylalanine, L=leucine, I=isoleucine, V=valine). (From Fernstrom and Wurtman, 1972).

Sex differences, 5-HT function, and appetite

Because of the large sex ratio discrepancy in eating disorders (see section 1.3.1), it is interesting and important to study female populations in order to further understand how normal eating behaviour may become disordered. Furthermore, with regard to 5-HT function, existing literature demonstrates some important sex differences which are relevant to an understanding of how manipulations of brain 5-HT function may have a greater impact on appetite in female populations.

This literature arose from the findings that female rats are more susceptible to stress induced hyperphagia in that their eating behaviour fails to normalise compared with males (Donohoe et al, 1987; Kennett et al, 1986). In addition, it has been shown that chronic fenfluramine infusion is more hypophagic in female rats than males (Rowland et al, 1986). Furthermore, administration of RU24969, and mCPP produces more hypophagia in females, although this is only seen with 24 hr food deprived animals, not with free feeding (Haleem, 1988; Clifton et al, 1993). A further demonstration of a sex difference comes from a study of chronic systemic administration of 5-HT to rats. Here it was found that both male and female rats lost weight compared with controls given only saline. Interestingly however, only the male rats demonstrated a decrease in food intake suggesting that the weight loss in females was a result of changes in metabolism (Edwards and Stevens, 1993).

1.6 SUMMARY AND GENERAL QUESTIONS TO BE PURSUED

Overviews of the current literature are presented in the above section. There is strong evidence for an involvement of 5-HT in appetite and mood, and perhaps in the pathophysiology of eating disorders. However, several questions arise from these reviews:-

i) Dieting. Because dieting is such a common behaviour, there is a clear concern for its role in the development of clinical eating disorders. It has been demonstrated that there is a causal link, but it remains unclear precisely how dieting can result in disordered eating patterns. It is also unclear why only a vulnerable minority of individuals is affected. There needs to be a fuller understanding of these mechanisms, along with a consideration of whether there are associated changes in mood states, a problem commonly seen with eating disorders. Prospective studies with adequate control groups are required to address these issues.

The demonstration of alterations in plasma tryptophan through dieting are particularly important because it suggests a possible mechanism through which dieting can alter of mood and appetite; that is, tryptophan depletion results in alterations in central 5-HT function, which in turn may produce abnormalities in appetite and mood. This may be particularly relevant to eating disorders because of the sexual dimorphism seen in the effects of dieting on brain 5-HT function, in that dieting-induced behavioural changes may be more susceptible to dysregulation in female populations. The depletion in plasma tryptophan seen in dieting has not been documented over time, and it would be important to describe the nature of these reductions in order to shed light on how this may interact with alterations in mood and appetite.

ii) Tryptophan depletion. As mentioned previously, it is important to establish a link between dieting behaviour and tryptophan depletion. In addition to this it would be interesting to pursue the relationship between acute reductions in plasma tryptophan and changes in mood and appetite. Evidence suggests that changes in these behaviours are seen in healthy male populations, but with the preponderance of eating disorders in females, and the suggested susceptibility of females to alterations in central 5-HT function, it is important to establish the effects of tryptophan depletion in female populations.

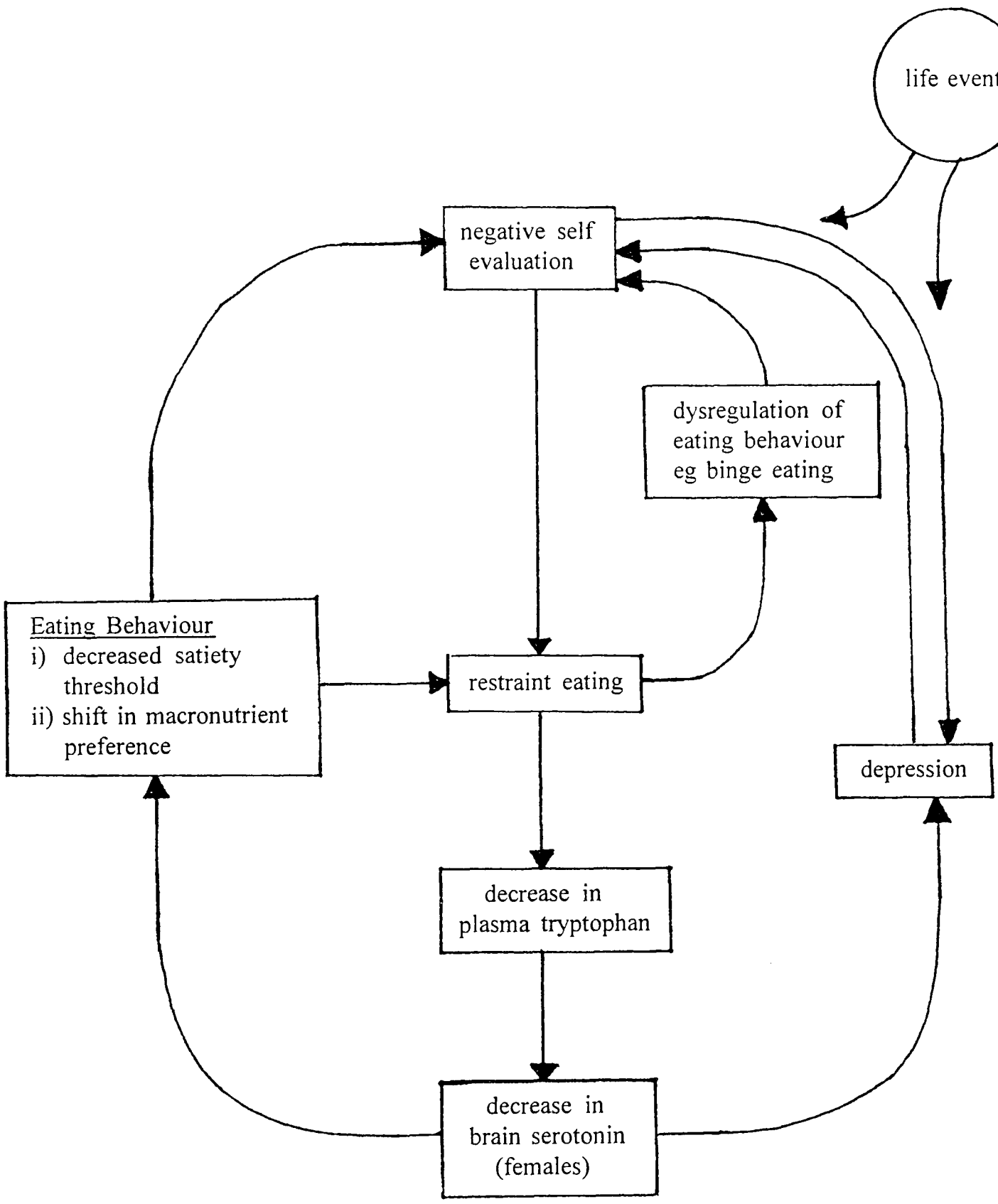
iii) Tryptophan depletion in recovered bulimics. It seems clear that there is a subgroup of people vulnerable to develop an eating disorder, and it would be of interest to examine the nature of this vulnerability. Research to date has documented biochemical changes in the acute stages of the disorders, and to a lesser extent after short-term recovery. Evidence suggests a role for 5-HT in both anorexia and bulimia nervosa, and it may be that abnormalities seen here are a reflection of an ongoing vulnerability. Few attempts have been made to examine the nature of likely mechanisms, and to offer models of how these disorders may develop; sociocultural models describe the pressures that exist within society to create a drive for thinness, but psychological theories have not offered a viable model to explain why some individuals will develop a disorder, whilst others do not. Biological theories may have more to offer in this area. From the review of eating disorders and dieting behaviour, it seems possible that depletion of plasma tryptophan may be an important aspect of this vulnerability. This could be answered by looking at long-term recovered eating disordered populations using methods to deplete plasma tryptophan.

Fig 1.6 is a model of how psychological and biological variables may interact to cause disordered eating. Several experimental chapters in the current thesis will test different

aspects of this model.

iv) Subreceptors of 5-HT and appetite regulation. If dietary changes in the supply of tryptophan to the brain for neurotransmitter synthesis result in alterations of brain 5-HT function, it would be important to reach an understanding of the nature of these alterations. It is possible that receptors may play a role in the development of eating disorders. Currently, very little is known about the way in which 5-HT receptor subtypes control appetite in humans. Preliminary work has been carried out with animal models, but it is unclear the extent to which these may apply to human populations. It would therefore be helpful to first establish which receptor subtypes are important in the control of appetite in humans.

Fig 6.1 An interactive model of cognitions, behaviour and 5-HT in disordered eating.



CHAPTER 2 GENERAL METHODS

2.1 TRYPTOPHAN DEPLETION METHODOLOGY

2.1.1 PILOT STUDY TO EXAMINE EFFECTS OF 50G AMINO ACID LOADING ON PLASMA TRYPTOPHAN

INTRODUCTION

Studies using large doses (100g) of amino-acid (AA) mixtures free of tryptophan have been carried out in normal and clinical populations (eg Young et al, 1985). It is well established that this dose lowers plasma total and free tryptophan (see section 2.1.3).

This method of acutely lowering plasma tryptophan has been used to examine predominantly male populations in investigating the effects of decreasing 5-HT function on mood, appetite, aggression, and pain perception (eg Delgado et al, 1990; Young et al, 1989). However, this procedure often results in side-effects such as nausea. It was therefore clear that this would not be a suitable dosage for measuring human behaviour, particularly that relating to appetite and food intake. A study was therefore carried out on a population of males and females in order to establish whether a lower dose of the essential amino acids would also lower plasma tryptophan. Tyrosine was added to prevent noradrenaline and dopamine depletion. The aim of this study was therefore to examine the efficacy of a lower dose of amino acids in lowering plasma tryptophan.

METHOD

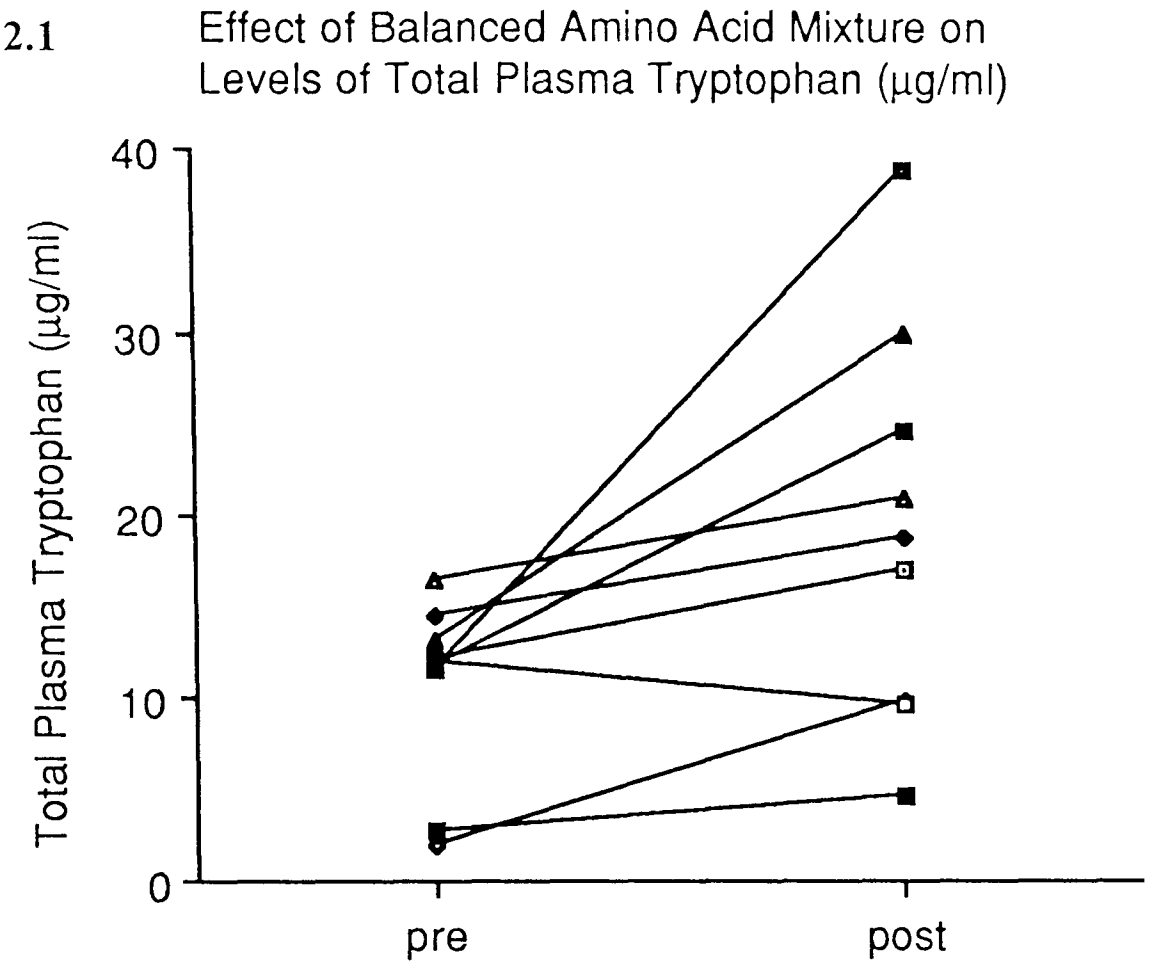
9 healthy volunteers (5 females, 4 males) were recruited for a double-blind, within subjects, crossover design study. Subjects received a balanced drink of the essential amino acids (excluding tryptophan) in the ratio present in garden peas. The quantities were: isoleucine 7g, leucine 10g, lysine 8g, methionine 2g, phenylalanine 6g, threonine 6g, and valine 7g. Tyrosine 6g, was added to make a total dose of 52g. A control drink containing an additional 2g tryptophan was given on a separate occasion. These drinks were flavoured and presented in the way described in section 2.1.3. Each subject's test sessions was separated by 1 week. Both test sessions were conducted in the follicular phase of the menstrual cycle with female subjects.

Blood samples were taken at 0, +3, and +4.5 hrs following ingestion of the drink, and were later assayed for total and free tryptophan by the method of Bloxam and Warren (1974), and Bloxam (1977). Differences at +4.5 hrs from baseline in both conditions were analyzed using paired t-tests.

RESULTS

4.5 hrs after ingestion of the drink, mean $\pm$ SEM plasma total and free tryptophan had fallen sharply. This was in contrast to the control drink where these measures were elevated (Figs 2.1 and 2.2). Means and SEMs are presented in Table 2.1.

Fig 2.1


Effect of Balanced Amino Acid Mixture on
Levels of Total Plasma Tryptophan (µg/ml)
Total Plasma Tryptophan (µg/ml)
40
30
20
10
0
pre
post

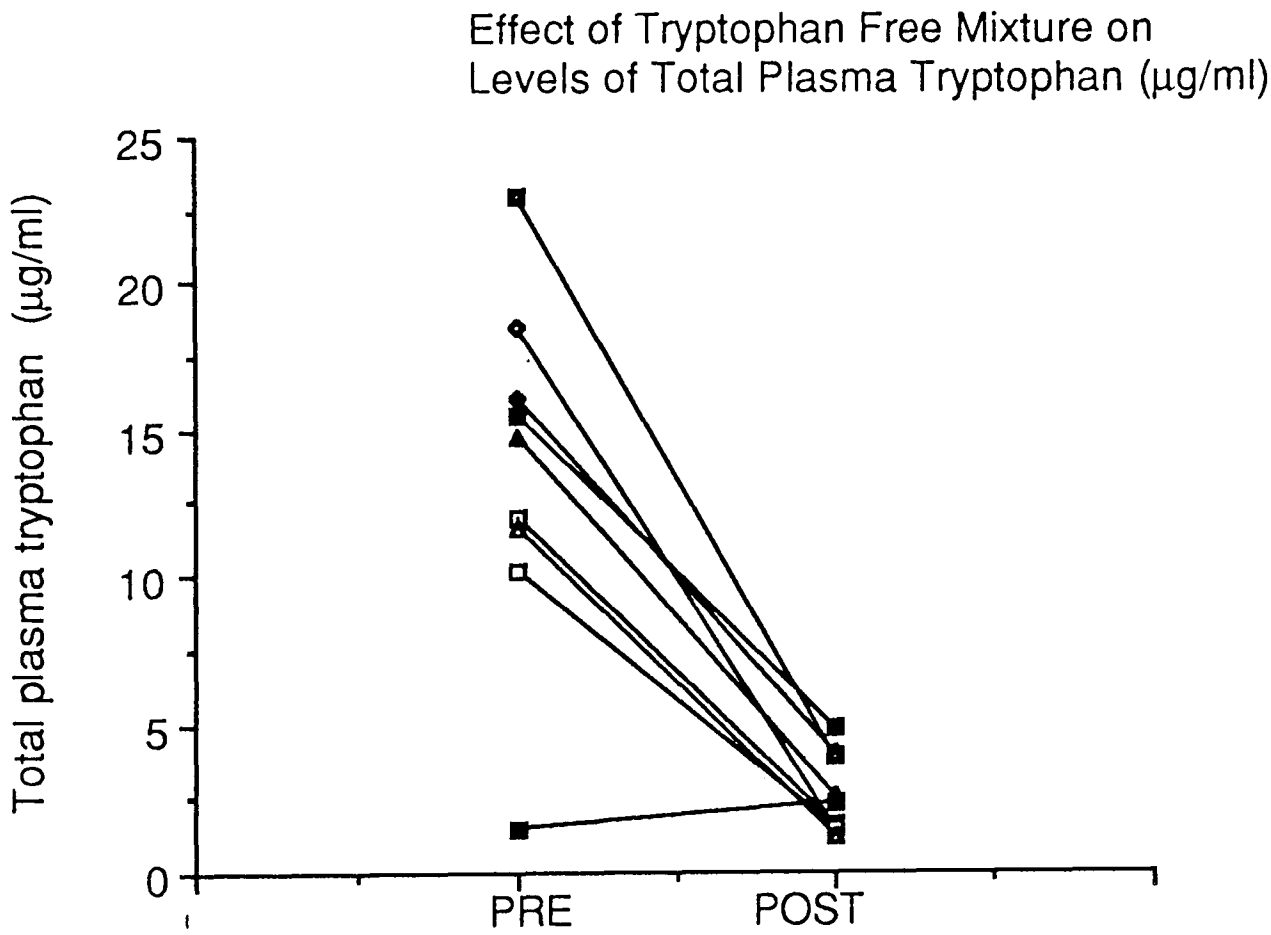
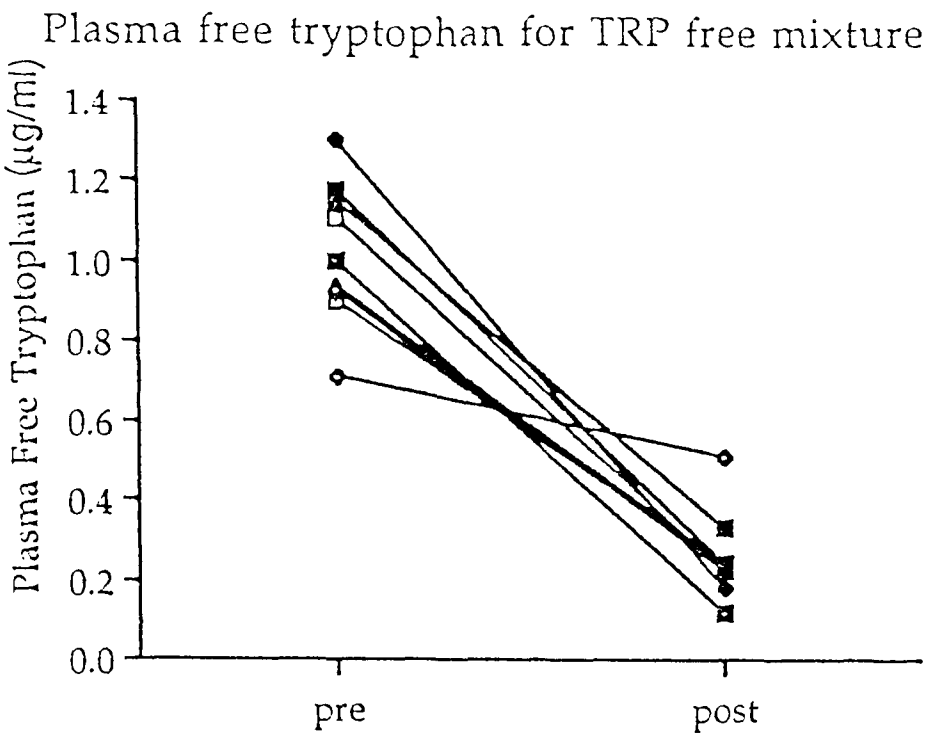

Effect of Tryptophan Free Mixture on
Levels of Total Plasma Tryptophan (µg/ml)
Total plasma tryptophan (µg/ml)
25
20
15
10
5
0
PRE
POST

Fig 2.2


Plasma free tryptophan for TRP free mixture
Plasma Free Tryptophan (µg/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pre
post

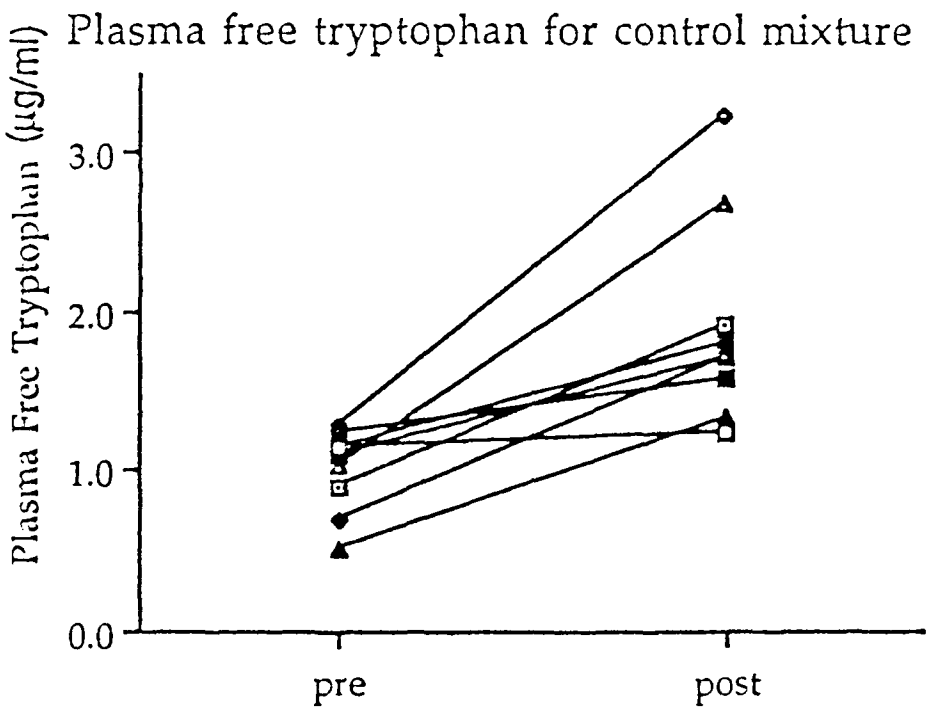

Plasma free tryptophan for control mixture
Plasma Free Tryptophan (µg/ml)
3.0
2.0
1.0
0.0
pre
post

Table 2.1 The Effects of Tryptophan Depletion on Plasma Levels of Total and Free Tryptophan, and on Branched Chain Amino Acids

		pre $\bar{x}$ (± SEM)	post $\bar{x}$ (± SEM)	P
Total plasma TRP µg/ml	TRP-free	13.64 (1.99)	2.59 (0.44)	0.0003
	Control	10.77 (1.67)	24.14 (4.24)	0.02
Plasma free TRP, µg/ml	TRP-free	1.02 (0.06)	0.26 (0.04)	0.0001
	Control	1.01 (0.08)	1.9 (0.21)	0.001
Branch chain amino-acids µM	TRP-free	376.45 (43.12)	1402.45 (116.93)	0.0001
	Control	419.33 (45.52)	1335.89 (156.99)	0.001

DISCUSSION

The data suggest that administration of a 50g amino acid dose is an effective method of lowering plasma tryptophan; the fall in both total and free tryptophan are comparable with changes reported by Young et al (1985, 1989). The 52g mixture was well tolerated by all subjects.

This lower dose of amino acids could therefore afford a useful and effective method of lowering plasma tryptophan in order to investigate the relationship between tryptophan depletion and psychological functioning. We subsequently decided to adopt the mixture used by Young et al (1985) for greater comparability of data and consistency of methodology, but to adopt this lower 50g dose which has been shown to be equally effective in reducing both plasma total and free tryptophan.

2.1.2 TRYPTOPHAN DEPLETION - INTRODUCTION

The activity of 5-HT pathways in the human brain can be lowered either by blocking 5-HT synthesis, or by restricting dietary tryptophan. Para-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, has been used in a number of human studies. For example, the effects of PCPA have been examined in depressed patients in order to elucidate the role of 5-HT in the action of antidepressant drugs (Shopsin et al, 1975 and 1976). However, this drug is associated with side effects including a high incidence of allergic eosinophilia, rendering its use as an investigational agent highly problematic (Miller et al, 1992). Alternative methodologies for depleting 5-HT include lowering the availability of tryptophan to the brain either by sustained dietary restriction, or by acute dietary

manipulation. Few studies of dietary restriction have been carried out in human populations because of the complexity of administering a diet low in tryptophan specifically. However, a number of studies of acute dietary manipulation have been carried out. This technique involves using a mixture of amino acids deficient in tryptophan in order to achieve an acute lowering of plasma tryptophan, and a reduction in its transport into the brain (eg, Young et al, 1985, Delgado et al, 1990).

The mechanism of this effect is as follows. First, tryptophan crosses the blood-brain barrier by an active transport mechanism which also serves as a carrier for five other large neutral amino acids (LNAA). Competition for the carrier site can lead to a deficiency of any of these amino acids relative to the others (Gessa et al, 1974). Thus, if a large dose of tryptophan deficient amino acids is given, the ratio of tryptophan to other LNAAs is reduced, restricting the amount of tryptophan that crosses the blood-brain barrier. As outlined in section 1.1.2, it is the supply of tryptophan to the brain which is the rate limiting factor in the synthesis process; hence decreasing tryptophan transport into the brain should lower brain 5-HT synthesis. Second, the mixture of amino acids, like any protein meal, induces the synthesis of hepatic protein, and subsequently reduces tissue and plasma tryptophan stores (Biggio et al, 1974). The amino acid loading therefore acts both to deplete plasma tryptophan via protein synthesis, and to limit the transportation of tryptophan across the blood-brain barrier.

The technique of amino acid loading has been shown to significantly lower levels of plasma total and free tryptophan in humans (Young et al, 1985; Delgado et al, 1990). Furthermore, animal studies have strongly supported the hypothesis that manoeuvre this also lowers brain tryptophan and synthesis of 5-HT. For example, Biggio et al (1974) demonstrated a 90%

lowering of free serum tryptophan, an 85% lowering of brain tryptophan, a 58% lowering of brain 5-HT and a 76% lowering of brain 5-HIAA two hours after administration of a tryptophan deficient amino acid load. It has further been demonstrated that a 31.5g dose of amino acids will lower 5-HIAA in CSF of vervet monkeys whilst levels of tyrosine and catecholamine metabolites remain unchanged (Young et al, 1989). A similar amino acid treatment decreases the 5-HT release evoked by electrical stimulation of the dorsal raphe nucleus of rats in vivo (Gartside et al, 1992a).

In conclusion, this technique of amino acid depletion provides an acute manipulation of brain 5-HT which can safely be carried out in human population. Studies undertaken in humans have generally used doses of 100g of amino acids (Young et al, 1985; Smith et al, 1987; Delgado et al, 1990). However, this dose is associated with nausea and other side effects, suggesting that a lower dose could be more appropriate. The pilot study carried out (section 2.1.1) established the efficacy of a lower dose of amino acids. Establishing that this technique is efficacious, a greater range of amino acids were used in subsequent studies in order that findings would be comparable with Young et al (1985, 1989). Thus, a lower dose was combined with the amino acids being present in the same ratios as the mixture employed by Young. The methodology adopted for the experimental chapters in the present thesis is outlined in section 2.1.3.

2.1.3 METHOD

Amino acids were presented to subjects as flavoured drinks. The experimental drink consisted of 15 amino acids in the ratio of those found in human milk. These are presented in table 2.2. Aspartic acid and glutamic acid were omitted because of concerns about their toxicity. A control mixture was made up of the same composition of amino acids with the

addition of 1.15g of tryptophan. This mixture was designed to be nutritionally balanced. Thus, these mixtures were similar in composition to those described by Young et al (1985), differing only in that they were made up to be exactly half the dose of the 100g drink.

TABLE 2.2: COMPOSITION OF AMINO ACID MIXTURE	
L- Alanine	2.75g
L-Arginine	2.45g
L-Cysteine	1.35g
Glycine	1.6g
L-Histidine	1.6g
L-Isoleucine	4.0g
L-Leucine	6.75g
L-Lysine monohydrochloride	5.5g
L-Methionine	1.5g
L-Phenylalanine	2.85g
L-Proline	6.1g
L-Serine	3.45g
L-Threonine	3.45g
L-Tyrosine	3.45g
L-Valine	4.45g
[L-Tryptophan]	[1.15g]

The final weight of the mixture was 51.25g with the addition of 1.15g of tryptophan for the control. This was then made into a drink using 300ml of refrigerated water, and the resulting suspension was flavoured with 12.5mg of saccharin and blackcurrant. This drink was made immediately prior to consumption, and administered in a standard drinking glass with straws in order to minimise the taste. Subjects were allowed to rinse out their mouths after the drink and chew on sugar free gum for 30 minutes.

2.2 MEASURES OF APPETITE

The studies carried out in the present thesis have combined two key methodologies in the measurement of appetite. This includes measures of total calorie intake, macronutrient intake (carbohydrate, protein, fat) which are calculated from test meal (section 2.2.1), and self-rated scales of appetite related parameters and macronutrient preferences (section 2.2.2).

2.2.1 TEST MEAL

The test meal is designed to measure total calorie intake (calculated in kJ), and the calorie intake of each macronutrient (carbohydrate, protein and fat).

Selection of items. During the initial screening session, all volunteers completed a food item checklist (Appendix 2.1). This asked volunteers to rate food items on a scale of 1-10 where 1 = 'dislike extremely', and 10 = 'like extremely'. The test meal lunches were then made up on the basis of these preferences. There were 7 different food categories on this list, and test meals included items from each category, plus margarine and tomatoes. This ensured a good selection from each type of macronutrient. The quantities of each item used

to make the meal are presented in appendix 2.2. For instances where some or all of the food items in one category had been rated equally, the following rule was adopted: if there was not a clear favourite item, the first item on the list was selected (eg milk chocolate for chocolate category). For sandwich fillings, the same rule was adopted. Here the top four items are chosen working down the list. However, there had to be at least two protein items in the final selection in order to ensure a balanced choice of macronutrients. These were cheddar cheese, cottage cheese, ham, tuna, chicken roll. If the final selection did not include two protein items, then the least preferred non-protein choice was swapped for the most favourite remaining protein item, and again if necessary.

Amounts. Each portion was presented to an excess in order to minimise the chance that constrictions are placed on the amounts consumed of any particular item. Quantities with approximate weights are set out in Appendix 2.2. Quantities remained constant, (eg two apples), and amounts therefore fluctuated slightly. Portions for male volunteers in the amphetamine study are larger, and are set out in Appendix 2.3.

Presentation. All excess packaging was removed (eg yoghurt pots, lids, crisp packets, tins), and foods were presented in plates and dishes which remained identical for all experiments. This minimised use of visual cues in the determination of how much of a particular item was consumed. Foods were presented in a standardised preset lay out on two trays. Subjects were also presented with drinking water.

Instructions to subjects. The following instructions were given to subjects: 'Ignore any dietary rules that you may have set yourself regarding the types of foods that you would/would not eat, and the quantities of foods that you would/would not eat, and to eat

until you have had enough'. Time taken to eat the meal is also recorded. This is done using a small electronic stopwatch placed at the back of the table. Volunteers are requested to press the stop button on this as they leave the room.

Calculation of food consumed. All food items were pre-weighed to the nearest 0.1g during preparation of meal using electronic scales (Ohaus, model CT1200-S, sensitive to nearest 0.01g). To prevent dehydration altering the weight of food, prepared items were covered with clingfilm and stored in the refrigerator. All weights were entered onto the record sheet (Appendix 2.4). Foods were weighed again to the nearest 0.1g immediately after consumption of the meal. Final weights were entered onto the record sheet.

Calculation of calorie consumption. The amounts of each food item consumed were calculated by subtracting final weight from initial weight. Using 'Direct', a specifically designed software programme, these data were then subjected to analysis in order to determine total calories consumed, total calories of fat, carbohydrate and protein, and total calories of sweet and savory foods. This programme runs an analysis for each meal separately, and provides a printout of the breakdown data for that meal (Appendix 2.5).

2.2.2 VISUAL ANALOGUE SCALES AND FOOD PREFERENCE QUESTIONNAIRES

Visual Analogue Scales (VAS). Visual analogue scales were used to measure various parameters of appetite and satiety, such as 'wish to eat', and 'hunger'. These consisted of 10cm lines with a scale of 0-100 across the top in increments of 10. Subjects were instructed to either place a mark on the line, or circle the number which best indicated how

they felt. For the test meal, these instructions required people to rate how they felt **at that moment**. VAS appetite scales were related to normal aspects of appetite and satiety. These are hunger, wish to eat, feel full, find/found meal satisfying, could eat a large amount of food. These are set out in appendix 2.6. Additional VAS's for specific studies will be set out in the relevant chapters.

Food Preference Questionnaires. Food preference questionnaires consisted of a 32 item free choice, and a 25 pair forced choice questionnaire from Blundell, Rogers, and Hill (Hill, 1987). Items which were common, and commercially available had been selected, and where possible, matched for energy content. Food items (excluding low energy items) therefore fell approximately within the range of 750-920 kJ.

Free Choice Checklist. This consisted of 32 items (eight carbohydrate, eight protein, eight fat, eight low energy). In order to be classified as of a particular macronutrient, each food item must contribute at least 50% of its total energy in the form of the macronutrient by which it is categorised. With the exclusion of protein items, these categories were represented by four sweet and four savory items. Appendix 2.7 contains the free choice checklist, and Appendix 2.8 contains a table of the nutritional breakdown of the food items included in this scale. Subjects were instructed to place a mark in the column by those items that they would wish to eat if placed in front of them **at that moment**. Subjects were asked to rate each item independently, ie to ignore any items they may have previously selected when making their decision.

Forced Choice Checklist. This measure was designed to examine the preference for protein items over carbohydrate lists, and consists of 25 pairs of foods, where each protein

item is paired with a carbohydrate item. This measure includes five high carbohydrate item, and 5 high protein items in all possible combinations of pairs. Subjects were instructed to chose one item from each pair, ie the one they would most like to eat at that moment in time (even if in some circumstances they did not really wish to eat either). (Appendix 2.9).

Presentation of appetite rating scales. VAS's and food preference checklists were given in a fixed order before and after the test meal. These were presented 10 minutes before and 10 minutes after completion of the meal.

2.3 MEASURES OF MOOD

Mood was assessed using VAS's and the Profile of Mood States Questionnaire (POMS) (McNair et al, 1971). In a similar fashion to appetite scales, subjects were required to complete these scales according to how they felt **at that moment**. Mood VAS are presented in Appendix 2.10.

Profile of Mood States Questionnaire (POMS). The 65-item version of the POMS was used to measure mood. This scale measures six mood or affective states: anxiety/tension, depression, anger/hostility, vigour, fatigue, and confusion. This is a standardised adjective rating scale (EdITS/Educational and Industrial Testing Service, 1981), and is a sensitive measure for use in normal, non-psychiatric populations. Subjects were required to rate their current mood state by circling the number which best applied to how they felt from 0 = not at all, to 4 = extremely. These scores were then tallied to give the six subscale totals.

2.4 BIOCHEMICAL MEASURES

Plasma total tryptophan

Plasma total tryptophan was assayed according to the method described by Bloxam and Warren (1974). 10μl volumes of plasma were added to 2ml of ice-cold 10% (W/V) trichloroacetic acid (TCA) contained in 4ml LP4 tubes. This was centrifuged at 2500g for 15 minutes at 4°C to remove protein. Standards in the range 0-2 nmoles tryptophan/tube were prepared similarly. To each sample stored on ice was added 0.2ml of 2% (w/v) formaldehyde and 0.1ml of 6mM ferric chloride in 10% TCA. The tubes were then vortex-mixed and immediately transferred to a heated dry block (temperature 100°C) for one hour. The fluorescence in each tube was measured in a Hitachi model 2000 spectrofluorimeter (excitation wavelength 300nm, emission wavelength 440nm).

Results were interpolated from standard curves constructed of fluorescence versus tryptophan concentration. Plasma tryptophan concentrations are quoted as micrograms per millilitre (μg/ml). Intra- and inter-assay coefficients of variation were 4.4% and 9.4% respectively and the limit of detection was 0.1μg/ml.

Plasma free tryptophan

Plasma free tryptophan was assayed according to the method described by Bloxam et al (1977). Using Millipore ultrafree 10,000 NMWL filters, 200μl volumes of plasma were pipetted into the filter reservoir. The sample was gassed with a 95% oxygen/5% carbon dioxide (CO_2) mixture by inserting a 19 gauge syringe needle into the reservoir above the

sample for 30 seconds before quickly closing the top of the centrifuge tube to maintain the gas atmosphere. This prevents plasma pH increasing during centrifugation due to loss of CO_2 (which would result in a change in the proportion of tryptophan bound to albumin). The tubes were then inverted for 10 minutes before being spun for 30 minutes in an eppendorph benchtop centrifuge.

The filtrate was analyzed for tryptophan as described above using all of the filtrate (approx 130μl) in the initial deproteinisation step.

2.5 A NOTE ON MENSTRUAL CYCLE AND THE STUDY OF APPETITE

This thesis pursues the question of how alterations in appetite may be linked to disordered eating, and clinical eating disorders. These studies are therefore largely carried out in female populations, and special attention needs to be paid to alterations in food consumption across the menstrual cycle in order that these fluctuations can be controlled for in the design of the experiment. The following review includes those studies which have documented the nature of these changes, and is taken largely from that of Goodall and Silverstone (1993).

Most studies have looked at the follicular (FOL) versus the luteal (LUT) stage of the cycle, where follicular is defined as the first 10 days of the cycle including menses, and luteal is defined as the last 10 days. Two studies have further differentiated a periovulatory phase.

Summary of findings (Goodall et al, 1993; Goodall et al, 1991b):

EFFECTS OF MENSTRUAL CYCLE ON ENERGY INTAKE	
AUTHOR	FINDING
DIARY STUDIES	
Dalvit-McPhillips, 1983	Increased intake LUT
Manocha et al, 1986	Increased intake LUT
Anantharaman-Barr et al, 1988	Increased intake (LUT) (late foll. vs premenstrual week)
FOOD WEIGHING STUDIES	
Tarasuk et al, 1991	Increased intake LUT
Lissner et al, 1988	No change
Gong et al 1989	Increased intake LUT (vs FOL and periovulatory)
Lyons et al, 1989	No change LUT Decrease periov. (vs premenses, post ov., and menses)
Czaja, 1978 (MONKEYS)	As above
Rosenblatt et al, 1980 (MONKEYS)	As above

SINGLE DAY COMPARISONS	
Pliner et al, 1983	Increased intake LUT
Leiter et al, 1987	Increased intake LUT
Bowen et al, 1990	Increased intake LUT
TWO DAY COMPARISONS	
Wurtman et al, 1989	No change

EFFECT OF MENSTRUAL CYCLE ON MACRONUTRIENT SELECTION	
DIARY STUDIES	
Dalvit-McPhillips, 1983	Increase CHO LUT
Anantharaman-Barr et al, 1988	Increase FAT LUT
SINGLE DAY COMPARISONS	
Leiter et al, 1987	Increase CHO LUT
Bowen et al, 1990	Increase CHO LUT
FOOD WEIGHING STUDIES	
Tarasuk et al, 1991	Increase FAT LUT
Lyons et al, 1989	Decrease PRO periovulatory

Tryptophan administration across the menstrual cycle

One study has looked at the effect of tryptophan administration during different phases of the menstrual cycle (Hrboticky et al, 1985). Here four feeding sessions were compared (2 LUT, 2 FOL) across two menstrual cycles where two cross-over doses of tryptophan (3g) vs two placebos were given. There was no effect of cycle phase on either energy intake or macronutrient selection. Tryptophan failed to modify energy intakes and macronutrient selection during both phases of the cycle. However, there was a correlation between plasma tryptophan and intake suppression in the FOL phase of the cycle, suggesting that the behavioural effects of tryptophan are related to menstrual cycle phase.

Tryptophan Metabolism

Hrboticky et al (1989) have examined the effects of the menstrual cycle on the metabolism of tryptophan loads. Tryptophan (3g) vs Placebo were given in the same way as above across two cycles (LUT vs FOL). At +3hrs tryptophan administration, plasma kynurenine levels were 40% higher in the LUT phase compared with the FOL phase, and a 28% increase in urinary kynurenine excretion, thus suggesting that the catabolism of tryptophan via the kynurenine pathway is affected by the phase of the human menstrual cycle.

Summary. As can be seen from the above studies, alterations most consistently occur in the luteal, especially late luteal premenstrual) phase of the cycle where design of experiment has allowed for this comparison. This is characterised most robustly, as an increase in consumption of carbohydrate. On the basis of the above review, it would therefore be pertinent to exclude the premenstrual phase of the cycle when studying appetite.

Oral Contraception. Volunteers for the studies contained within this thesis were mainly women taking standard oestrogen- and progesterone-containing oral contraceptives. Because of the interaction between these hormones and brain 5-HT function, it must be acknowledged that this may have had some bearing on the findings presented.

CHAPTER 3 THE EFFECTS OF CALORIE RESTRICTED DIETING ON PLASMA TRYPTOPHAN, MOOD AND APPETITE

3.1 INTRODUCTION

Dieting has been shown to lower significantly both plasma tryptophan concentrations and the ratio of plasma tryptophan to other neutral amino acids (Anderson et al, 1989, 1990; Goodwin et al, 1990). These studies report a decrease in total plasma tryptophan in both sexes, and show a greater reduction in women in absolute and percentage terms. This effect exists despite there being no significant difference in the percentage weight loss between males and females (Anderson et al, 1990b). However, not all studies report this reduction (Goodwin et al, 1987). Plasma free tryptophan concentrations appear to be unchanged by moderate dieting (Anderson et al, 1990b), and concentrations of branch chain amino acids (BCAA's) are also unchanged (Anderson et al, 1989, 1990b). The ratio of total tryptophan to BCAAs is therefore reduced, thus decreasing the transport of tryptophan across the blood brain barrier.

Studies of neuroendocrine responses to challenge test using 5-HT probes show that this lowering of plasma tryptophan concentrations is associated with alterations in brain 5-HT function. These studies have demonstrated that dieting alters 5-HT mediated neuroendocrine responses in women, but not men. Volunteers have been tested with several neuroendocrine probes; Goodwin et al (1987) and Anderson et al (1990b) report enhanced PRL responses to L-tryptophan in female subjects, with no change in males. Walsh et al (in press) have shown enhanced PRL responses to d-fenfluramine in female dieters, but not males. As reviewed in section 1.4.3, these neuroendocrine alterations appear to be specific

to alterations in 5-HT, as dopamine mediated prolactin release is not altered by dieting (Anderson et al, 1989).

These findings are of interest because, taken together, they provide good evidence that dieting alters the supply of tryptophan to the brain, and that women are more vulnerable than men to the effect of precursor deficit on brain 5-HT synthesis and function.

In addition to the above findings, it is known that dieting is important in the development of eating disorders (section 1.4.2). From the review of prevalence rates of anorexia and bulimia nervosa, it can be seen that women are many times more at risk of developing these disorders than men (section 1.3.1). It is therefore of considerable theoretical importance to consider the potential role of dieting-induced alterations in brain 5-HT in the development of clinical eating disorders. Whilst it is not practical to examine the progression of normal eating behaviour to clinical disorder, it would be possible to adopt a longitudinal approach in order to examine alterations in appetite and eating behaviour. The model outlined in Fig 6.1 (page 100) suggests how restraint eating and the subsequent decrease in plasma tryptophan may lead to alterations in the mechanisms controlling appetite. Furthermore, this model suggests how dieting-induced decreases in plasma tryptophan may result in alterations in mood. Section 1.1.1 reviews the strong link between brain 5-HT function and mood. There is also much evidence to suggest that anorexia and bulimia nervosa are associated with alterations in both brain 5-HT function and in mood (section 1.3.2). Thus, the model suggests that alterations in both mood and appetite may be a consequence of dieting-induced alterations in plasma tryptophan and brain 5-HT function.

However, it can be seen from the studies reviewed above that the time course of alterations in plasma tryptophan remains unclear as comparisons were made before and after dieting, rather than across the whole of a dieting period. Furthermore, there were no data available from any control group, making interpretation of changes in tryptophan difficult. A fuller study of the time course of alterations in plasma tryptophan would be important in a theoretical consideration of how these may interact with mood and appetite regulation.

Whilst relatively little work has been carried out on the effects of dieting on psychological functioning, some evidence suggests that dieting is associated with increased reports of depression, irritability, and other mood states (Keys et al, 1950; Goodwin et al, 1987). There have, however, been negative reports in this area (Pirke et al, 1985; Warren and Cooper, 1988) (see section 1.4.1 for review).

The role of dieting in disordered eating has received more attention, and there are now clear associations between restraint and disordered eating (Herman and Polivy, 1988). Despite the clear role of neurotransmitter function in the control of appetite, little attempt has been made to offer a theoretical basis of how this may interact with psychological mechanisms. Section 1.5.2.3 reviews this literature from which it can be seen that a decrease in brain 5-HT function is associated with increased food consumption, involving an increased preference for carbohydrate over other macronutrients. As a result, a dieting-induced reduction in brain 5-HT function may predispose an individual to desire to eat more food, with a selective preference for carbohydrate items over other macronutrients. However, this increased urge to eat would be in direct conflict with their dietary aims of reducing their intake, perhaps predisposing the individual to binge eat.

Large numbers of the population attempt to follow calorie controlled diets (Hill et al, 1992), and increasing numbers of individuals are developing eating disorders (section 1.3.1) thus, this area of study is important.

The present study has three main objectives. First, to study the time-course and nature of tryptophan depletion in dieting females compared with controls. Second, to examine the effects of calorie controlled dieting on mood, and third, to examine the effects of dieting on behaviour related to binge eating and macronutrient preference. The design of the present study will allow for correlational analyses to be carried out in order to unravel the relationship between these behaviours and changes in plasma tryptophan.

The study adopts a prospective design, examining the effects of dieting compared with a matched control group, and overcoming many of the problems inherent in interpreting data from the studies of cross-sectional design. This study will therefore provide a main analysis of between subjects comparisons, with further correlational analyses where appropriate.

3.2 METHODS

Volunteers

Through advertisement, 32 healthy female volunteers were recruited to go on a three week calorie controlled weight-reducing diet. These volunteers were part of a larger study looking at the effects of dieting on neuroendocrine function. All volunteers were carefully screened to ensure that they were in good health, had no history of psychiatric disorder or eating difficulties, and no current dietary restriction. None of the volunteers had been dieting within the three months immediately prior to their inclusion in the study. All volunteers were within the normal BMI range (21.0 - 29.5, mean 24.5 SD 2.0). Ratings of abnormal eating as assessed using the Eating Attitudes Test (EAT) (Garner and Garfinkel, 1979) were within the normal range (0 - 18, mean 7.6, SD 4.11). Levels of depression were assessed using the Beck Depression Inventory (BDI) (Beck et al, 1961). All volunteers fell within the range of 0 - 16, (mean=3.23, SD=±3.37). The age of volunteers fell within the range of 20 to 38 years (mean=27.3, SD=±5.8). All women had a regular 28 day menstrual cycle. Groups did not differ on these measures as can be seen from table 3.1.

TABLE 3.1: GROUP ASSIGNMENT: MEANS AND STANDARD DEVIATIONS

MEASURE	DIET (MEAN ±SD)	CONTROL (MEAN ±SD)
BMI	24.53 ±1.47	24.45 ±2.53
BDI	3.31 ±3.98	3.14 ±2.66
AGE	27.08 ±5.80	27.56 ±6.01
EAT	7.43 ±4.47	7.73 ±3.84

Experimental Design

The experiment was a between groups, matched, open design. The two groups, dieters (n=16) and controls (n=16), were matched on age and BMI. This design enabled between groups comparisons on all measures, and within subjects comparisons on repeat measures. With the exception of the calorie restricting diet and the neuroendocrine challenges, both groups were examined in exactly the same way. The study was controlled for menstrual cycle with each subject entering in to the study at the same phase of the cycle (early follicular).

The Diet

The diet consisted of a daily allowance of 1000 kcals. Dieting volunteers were required to follow a prescribed diet which had been devised by the Department of Nutrition and Dietetics, John Radcliffe Hospital (II), Oxford. This provided dieters with a daily eating plan, advice on calorie content of foods and cooking methods, and a stipulation of the daily dietary allowance (Appendix 3.1). The daily allowance consisted of 0.5L skimmed milk; 20g of polyunsaturated margarine; two servings of either lean meat (60g), white fish (120g), cheese (30g), cottage cheese (90g), one egg, or one small carton of yoghurt; five slices of wholemeal bread; one serving of whole-grain breakfast cereal; and two portions of fruit.

Dieters were required to keep a daily diary of the foods eaten and their total calorie intake. This diary also included all daily visual analogue scale (VAS) ratings of mood (see below). Instructions of how to keep the diary were inserted (Appendix 3.2).

Measures

Plasma tryptophan. Fasting blood samples were taken at the end of each weekly session. This included two baseline samples taken at the beginning and end of the pre-diet week, and one sample for each diet week. Sample one was therefore matched with the final diet sample for day of menstrual cycle.

Mood. Volunteers were required to complete daily ratings of mood for the duration of the study (five weeks in total). These were rated on 100mm anchored VAS inserted into the food diary. Subjects were required to reflect over the day and make a rating for how they had been feeling, on average, for that day. The parameters were despondent/sad, concentration, energy, tension, anxiety, irritability (Appendix 2.10). In addition, at the beginning and the end of the study period, mood was assessed using the Beck Depression Inventory (BDI) which is designed to assess the previous week. For all BDI scores, question 19 (asking about weight loss) was omitted to keep the scores for the two groups comparable. Mood was also rated on a weekly basis using the Profile of Mood States Questionnaire (POMS) (McNair et al, 1971) at the research unit, though this data has not been analysed as a result of incomplete data collection.

Appetite. Appetite was measured using daily VAS ratings of hunger as above. In addition, subjects attended the unit for a lunchtime test meal both before dieting and at the end of the dieting period. (See section 2.2 for detail of protocol).

Eating disturbance. This was assessed using daily VAS's as above (Appendix 3.3).

These were completed for one week prior to dieting (pre-diet week), and for the week immediately after dieting (post-diet week). The parameters related to: preoccupation with thoughts of food, urge to eat, ability to stop eating once started, fear of loss of control, eating when not hungry, resisting the urge to over-eat.

An eating interview based on the Eating Disorders Examination (EDE-Q3) (Cooper and Fairburn, 1987) was carried out before and after dieting to examine changes in objective and subjective ratings of overeating (Appendix 3.4). This is a structured interview which yields data on total number of episodes of overeating retrospectively for the previous week. The subscales are: subjective overeating, objective overeating, subjective bulimic episode, objective bulimic episode (the latter referring mainly to eating behaviour in patient groups). The interview was carried out at the unit in a separate room by the same person. Full training for interview technique and assessment of data was received by an EDE interviewer on the Eating Disorders Project, Department of Psychiatry, Oxford.

Weight. Subjects were weighed weekly. This was carried out on arrival at the research unit while subjects were still fasting. Scales were manual Avery scales (type 3306 ABV).

Procedure

Group assignment. The dieting and the control groups were matched for both age and BMI such that each dieter had a matched control. Initially volunteers were allocated to either the diet group or control group in a random fashion, and subsequent volunteers were then randomly allocated to an appropriate match. Any volunteers who could not be matched

were not included in the study. Subjects were not informed of which group they would be entering until the end of their first test meal visit.

The study was controlled for menstrual cycle; the study phase began with the pre-diet week (early to mid follicular), and lasted a total of five weeks (pre-diet week, three diet weeks, post-diet week).

Subjects were required to attend the unit for a total of nine sessions in order to successfully complete the study. These were as follows:-

TABLE 3.2: DIET STUDY PROTOCOL

SESSION NO.	VISIT	ASSESSMENTS	PHASE OF CYCLE
1	Screening	EAT, BDI, BMI, lunch preference form for test meal	n/a
2	Test Meal (pre-diet)	VAS, POMS, food preference qu'aire, test meal	7-12
3	Weight 1 (beginning of pre diet week)	EDE-Q3 qu'aire, weight, blood, diary given, (neuroendocrine challenge for dieters)	7-12
4	Weight 2 (end of pre-diet week)	BDI, POMS, weight, blood, eating interview, food preference qu'aire for midday.	14-19
5	Weight 3 (end of first diet week)	POMS, weight, blood, food preference qu'aire for midday	21-26
6	Weight 4 (end of second diet week)	POMS, lunch preference form for test meal, weight, blood food preference qu'aire for midday	28-6
7	Test Meal end of diet	VAS, POMS, food preference qu'aire, test meal	7-12

8	Weight 5 (end of third week of diet)	POMS, BDI, EDE-Q3 qu'aire, weight, blood, food preference qu'aire for midday (neuroendocrine challenge carried out next day for dieters)	7-12
9	Weight 6 (end of post-diet week)	POMS, BDI, food preference qu'aire for midday, weight, eating interview	14-19

Assessment

Volunteers who contacted the research unit with a view to participation in the diet study were invited for an initial visit. On this visit volunteers were given a written and verbal explanation of the study, and of what their involvement would be. It was explained that the study consisted of a dieting group, and a control group, and that all volunteers would be randomly allocated to one of these groups. Any volunteers who did not agree to random allocation were excluded from this study. Volunteers were asked to complete a BDI, EATS, test meal food preference form. Instructions for how to complete these forms were given verbally as well as in written form. Volunteers were then assessed by a clinician; a personal and family medical and psychiatric history was taken. At this stage volunteers were excluded if they had a BDI score of greater than 16; an EAT score of greater than 20; any major physical disorders, or ongoing medical conditions (including asthma, allergies, etc); any personal history of psychiatric disorder; any family history of affective disorder; any eating difficulties which were felt to be risk factors (eg, over-concern with body weight

and shape, restrained eating). Because the food preference questionnaire contained meat items, vegetarians were excluded from the study. Volunteers who fell outside the normal range of weight for height were excluded. The upper limit for this range was BMI greater or equal to 30. The lower limit for this was BMI less than or equal to 20. The lower limit was calculated taking into account the expected weight loss through dieting (ie 3kg), ensuring that this would not cause weight to fall below the normal range. To ensure matching for menstrual cycle, any volunteer who did not have a regular 28 day cycle was excluded.

During the assessment visit, volunteers were given the opportunity to ask questions. It was emphasised that they were free to withdraw from the study at any time, and did not need to give an explanation for this. Preliminary dates and times were set up with the volunteer for the study visits, and volunteers were asked to phone to confirm these dates at the onset of their next menstrual cycle.

The Study

For the two test meal sessions, volunteers were required to fast from midnight of the night before until the test meal. They were asked to only drink water during the morning. These instructions were given again a few days prior to the test meal sessions. The test meal protocol is set out in section 2.2. Before the second test meal (session 6), subjects were given a second lunch preference form from which their second test meal would be made. This was to allow for changes in food preference across the dieting period.

For each volunteer, test meal sessions were at the same point in the menstrual cycle +/- 2 days.

At the end of test meal 1, subjects were informed of which group they had been allocated, and that they were to continue eating normally (ie follow their own normal eating behaviour) until the diet period began, or, for controls, for the study period.

Weights 1 to 5; subjects attended the research unit while fasting, and at a fixed time during the weight morning. The order of giving the rating scales and collecting the data was fixed, with psychological rating scales and questionnaires being completed first (see table 3.2), followed by weight assessment then blood sample. Finally, diaries were checked, and dietary advice given if necessary. The POMS and BDI questionnaires referred to the whole of the previous week; the EDE-Q3 referred to the previous 4 weeks. For the initial presentation of each of the questionnaires, verbal and written instructions were given.

Test meal and eating interview data were collected by the author, and all remaining data were collected by the nursing staff at the research unit.

On the final visit, volunteers were thanked for their participation in the study, and asked to complete an expenses form for reimbursement. Dieters were paid on a scale of £40 - £80 for successful completion of the study (ie minimum weight loss of 2kg, maximum weight loss of 3kg). Control dieters were paid £35 for successful completion of the study.

Biochemical Methods

Levels of plasma tryptophan were determined from 10ml fasting blood samples which were taken into heparin tubes. This was carried out on a weekly basis at the unit after weight assessment. Plasma samples were separated by centrifugation and stored at -30°C. Plasma total and free tryptophan were determined by fluorimetric detection according to the method of Bloxam and Warren (1974), and Bloxam et al (1977). (See section 2.4 for outline of methodology of assay procedure for total and free tryptophan).

Data Analysis

Group (diet vs control) data were analyzed by analysis of covariance (ANCOVA) using the BMDP p2v statistical package. Greenhouse Geisser probability was used for repeat measures. Correlational and chi-squared analyses were carried out on the SAS statistical package.

A two-tailed Fisher's exact test was used to analyze episodes of overeating as assessed using the eating interview.

3.3 RESULTS

The results of the biochemical and psychological data analyses are set out below. Means and SD's of key variables are set out in table form. For biochemical data, SEM's are presented to allow comparison with existing literature.

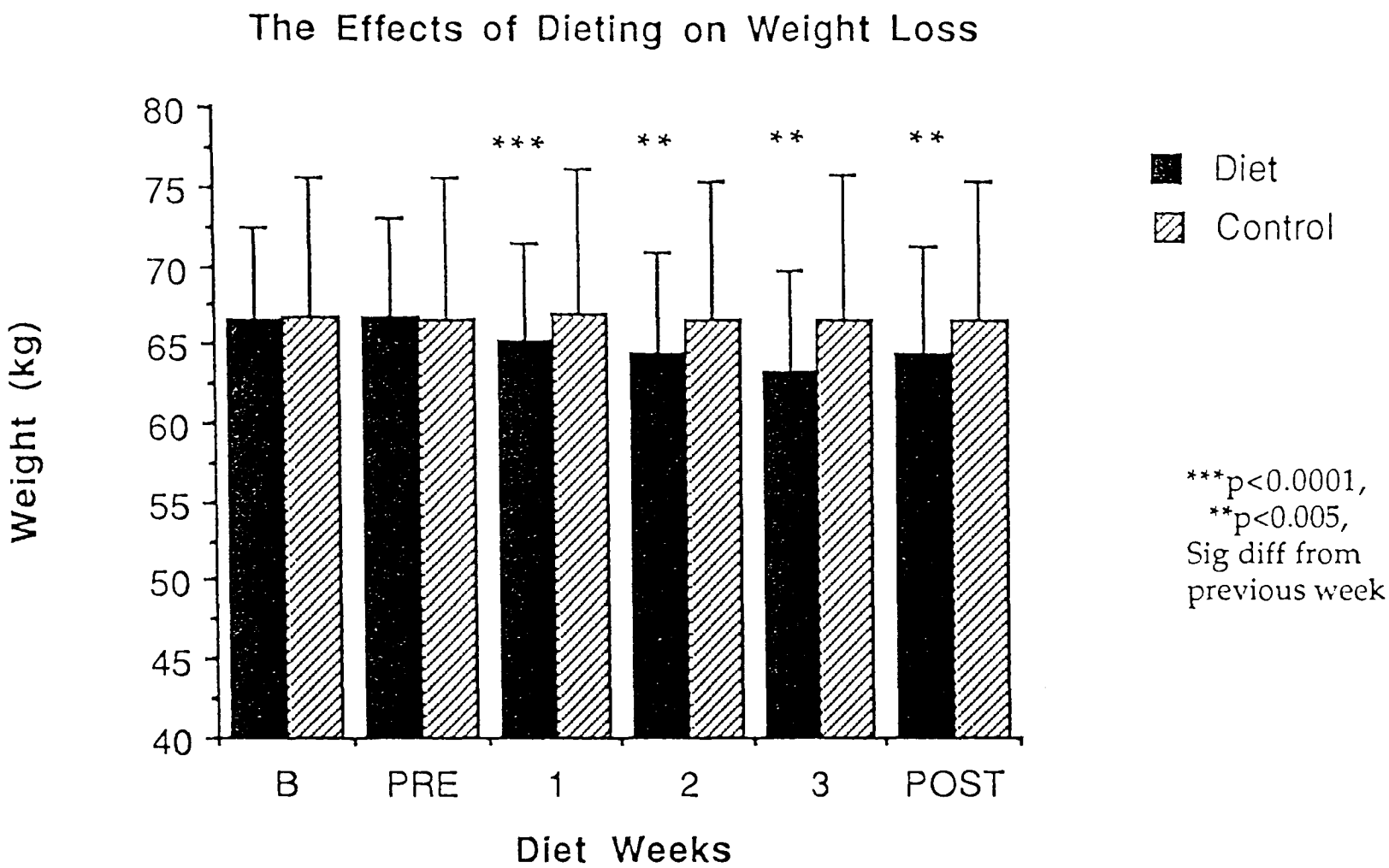
Manipulation Checks

Diary records of food consumption and weight loss were considered to check that all volunteers had complied with their instructions either to diet or to maintain normal food intake. Inspection of the diaries for both groups revealed that volunteers had conformed well to their dietary manipulation.

Weight

The ANCOVA revealed that dieting resulted in a significant weight loss; with a significant main effect of group ($F_{1,28}$=14.75, $p<0.001$), and time ($F_{4,116}$=19.07, GGp<0.00001). There was also a significant group by time interaction ($F_{4,116}$=17.61, GGp<0.00001) (Table 3.3 and Fig 3.1). Post hoc t-tests revealed that for the diet group this loss was significant for each diet week when compared with the previous one with a highly significant fall in the first week (df=15, t=12.8, $p<0.0001$), and with significant falls in the remaining two weeks (df=15, t=3.5, 3.7, $p<0.005$, respectively). The post-diet week showed a significant increase in weight in the diet group (df=15, t=-3.7, $p<0.005$). In comparison there were no significant changes in the control group between any of the study weeks when compared with the previous week with the exception of the second week of control diet where there was a small but significant decrease in weight (df=15, t=2.9, $p<0.05$).

Fig 3.1


The Effects of Dieting on Weight Loss
Weight (kg)
80
75
70
65
60
55
50
45
40
***
**
**
**
B
PRE
1
2
3
POST
Diet Weeks
Diet
Control
***p<0.0001,
**p<0.005,
Sig diff from
previous week

Biochemical Measures

Plasma concentrations of total and free tryptophan were analyzed for both groups across the pre-diet week and the three diet weeks. For plasma total tryptophan, the ANCOVA revealed a significant main effect of group ($F_{1,24}$=7.73, p=0.01) (Table 3.4). There was also a trend towards a group by time interaction ($F_{3,75}$=2.8, p=0.052), but no main effect of time ($F_{3,75}$=0.9, p>0.05). Because of the significance level of the interaction, post hoc unpaired t-tests were carried out (Fig 3.2). This trend towards an interaction appeared to be a result of significant group differences at the end of the first and second weeks of dieting. In these weeks dieters had significantly lower levels of plasma total tryptophan (df=25, t=2.3, p<0.05; df=25, t=3.5, p<0.005, respectively). For the final week of dieting, this difference was no longer significant.

The ANCOVA revealed no significant main effect of group on plasma concentrations of free tryptophan ($F_{1,24}$=0.52, p>0.05) (Table 3.4). There was no main effect of time ($F_{3,75=}$2.17, Ggp>0.05), or group by time interaction ($F_{3,75=}$0.84, Ggp>0.05). The means indicated a difference at baseline, but a t-test indicated that these differences were not significant (df=25, t=1.2, p>0.05) (Fig 3.3).

Appetite related measures

Measures of appetite were included firstly, to examine the effects of dieting on the satiety mechanism, and secondly, to correlate changes in plasma tryptophan with changes in appetite.

Fig 3.2

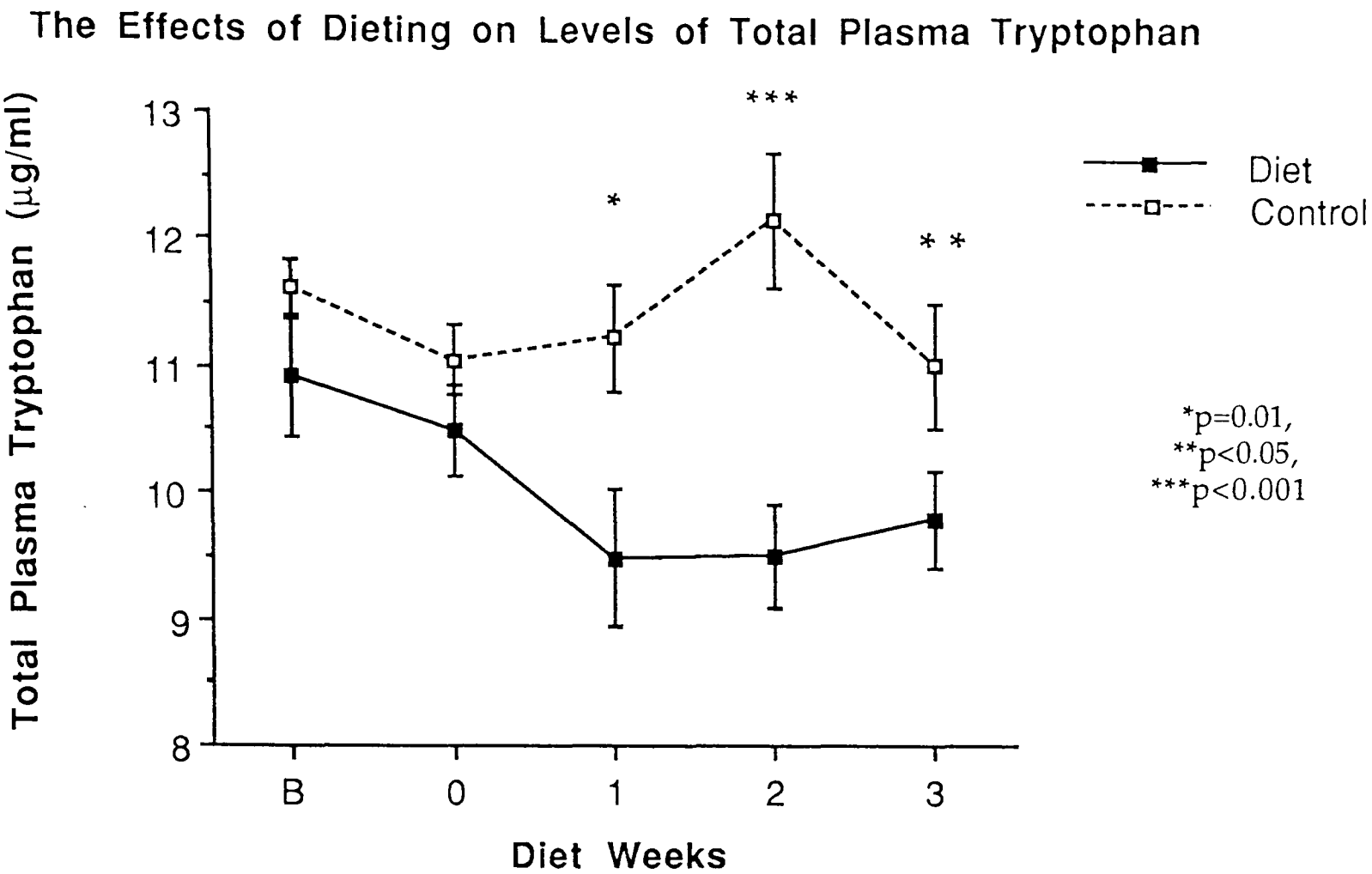
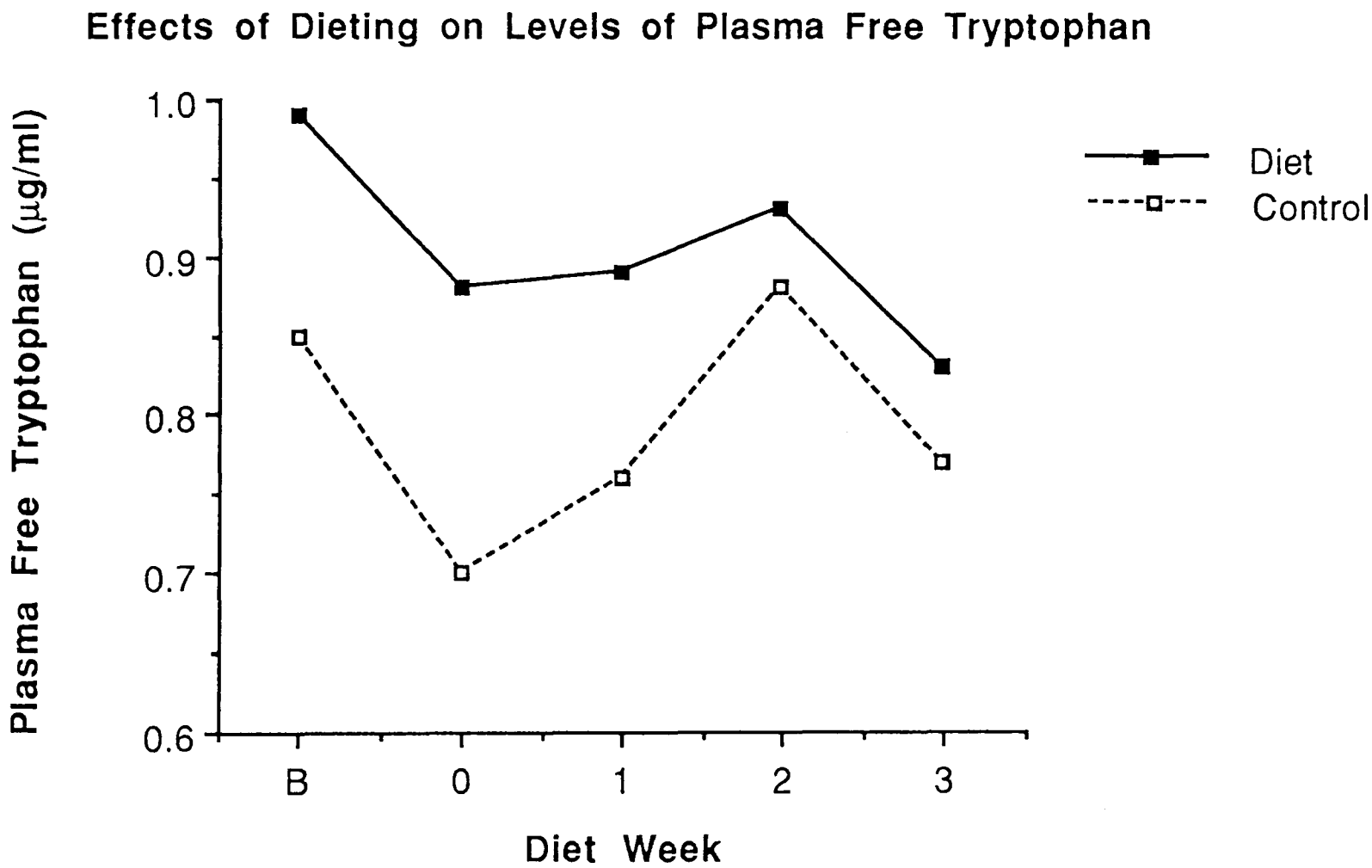

The Effects of Dieting on Levels of Total Plasma Tryptophan
Total Plasma Tryptophan (μg/ml)
13
12
11
10
9
8
B
0
1
2
3
Diet Weeks
*
***
**
Diet
Control
*p=0.01,
**p<0.05,
***p<0.001

Fig 3.3


Effects of Dieting on Levels of Plasma Free Tryptophan
Plasma Free Tryptophan (μg/ml)
1.0
0.9
0.8
0.7
0.6
B
0
1
2
3
Diet Week
Diet
Control

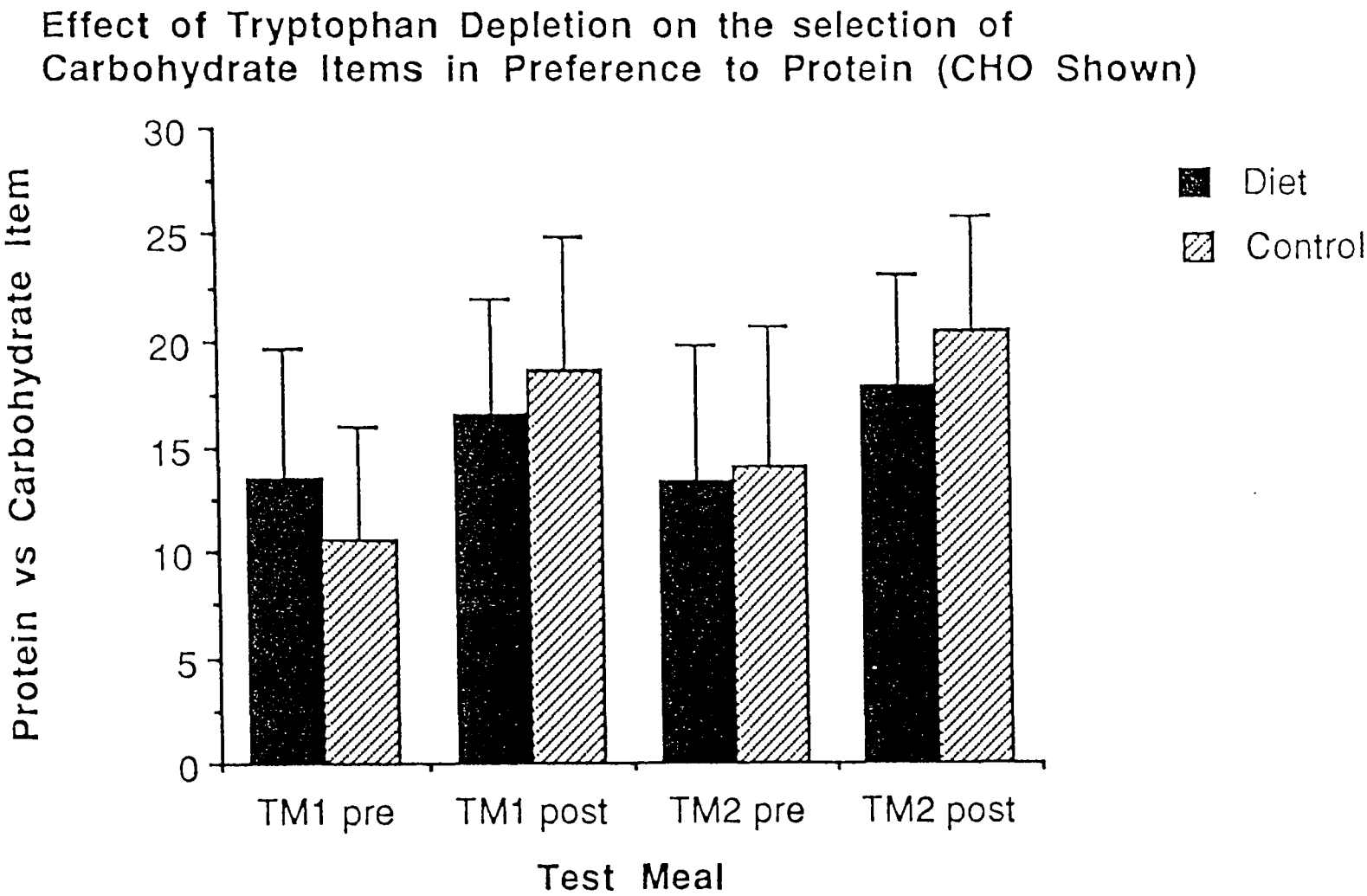
These measures were analyzed to examine the effect of dieting on both subjective and objective measures of appetite. VAS ratings of hunger across the 5 weeks of the study revealed no significant differences between the two groups (Table 3.5). There was no main effect of time, or group by time interaction.

Satiety related VAS at test meal; there was no change on these measures of satiety, with no main effect and no group by time interaction (Table 3.5). In addition to this, actual total number of calories consumed at test meal was not altered by dieting (Tables 3.5 and 3.6).

Macronutrient selection was assessed by analysis of foods consumed and food preference questionnaires at test meal. There were no changes in macronutrient selection (fat, carbohydrate, protein) as a result of dieting (Tables 3.5 and 3.7). Similarly, for the free choice food preference ratings there were no significant group by time interactions (Tables 3.5 and 3.7). For selection of carbohydrate items, however, there was a trend towards an interaction of group by time. This appeared to be coming from an increase in the selection of carbohydrate items post meal in the diet phase of the study (test meal 2) compared with the pre diet phase, and the control group who showed the converse pattern of selection.

With the forced choice questionnaire, there was a significant main effect of group (Tables 3.5 and 3.7), and of time ($F_{2,52}$=13.31, $p<0.0001$), but no group by time interaction (Fig 3.4). The group effect appeared to be a result of relatively decreased selection of carbohydrate items compared with protein items in the diet group compared with the controls. The effect of time was a result of increased selection of carbohydrate post meal in both groups.

Fig 3.4


Effect of Tryptophan Depletion on the selection of
Carbohydrate Items in Preference to Protein (CHO Shown)
Protein vs Carbohydrate Item
30
25
20
15
10
5
0
TM1 pre
TM1 post
TM2 pre
TM2 post
Test Meal
Diet
Control

Eating Disturbance

Eating interview and daily ratings of eating disturbance related VAS were analyzed in order to examine whether dieting exacerbated these behaviours. A 2-tailed Fisher's exact test was carried out on the eating interview data. This examined total numbers of people experiencing episodes of overeating. There were no significant differences between the groups in reported episodes of overeating.

There were no significant between group differences in people experiencing subjective overeating post-diet ($p>0.05$). There were also no pre-diet differences between groups. There were also no differences between groups in people experiencing subjective bulimic episodes either pre- or post-diet ($p>0.05$).

This pattern also held true when numbers of people experiencing any form of overeating were analyzed (ie all episodes summed); differences pre dieting were non significant for group ($p>0.05$), as were differences post dieting ($p>0.05$).

In addition to this, a closer analysis of the data indicated that there was no association of reported eating episodes on the first and second occasion, indicating a relatively random occurrence of overeating amongst people in the study. That is, although there was a non-significant increase in the number of people experiencing episodes of overeating, it was not the case that particular people got worse, but rather that overeating occurred in people who had not reported this at baseline, and vice versa, people who had experienced baseline overeating did not report this post-dieting. (For both dieters and controls, an analysis of total episodes pre and post-diet generated p values >0.05. Thus, the data suggest that there is no tendency for dieting to exacerbate overeating, and that behaviour pre-dieting is not

a predictor of post-dieting behaviour.

Pre- and post-diet VAS daily ratings of eating disturbance revealed a significant main effect of group for 'fear of loss of control of eating' and 'feeling unable to stop once started eating', but no group by time interaction (Tables 3.8 and 3.9). Examination of the means revealed that there were significant differences between groups for fear of loss of control during the post-diet week, where dieters experienced an increased loss of control across three days (Fig 3.5). This pattern held true for feeling unable to stop eating once started, which was significantly elevated in one of the post-diet days for the diet group (Fig 3.6).

There were non-significant trends for main effects for 'having strong urges to eat' (Fig 3.7), and for 'having to resist the urge to overeat' (Fig 3.8), but no group by time interactions. For both of these the trend was for the ratings to be higher in the dieting group post-diet, thus dieters reported stronger urges to eat and an increase in resisting the urge to overeat.

There was no main effect or group by time interaction for VAS ratings of 'preoccupation with thoughts of food' or 'eating at times when not hungry' (Tables 3.8 and 3.9)

Four of the above VAS parameters were measured in response to the test meals (ie 'preoccupation with thoughts about food', 'fear of loss of control of eating', 'feeling unable to stop eating', 'having to resist the urge to overeat'). Here there were no significant main effects of group, or group by time interactions suggesting that dieting did not alter subject's concerns about food when presented with a meal in a laboratory setting.

Fig 3.5

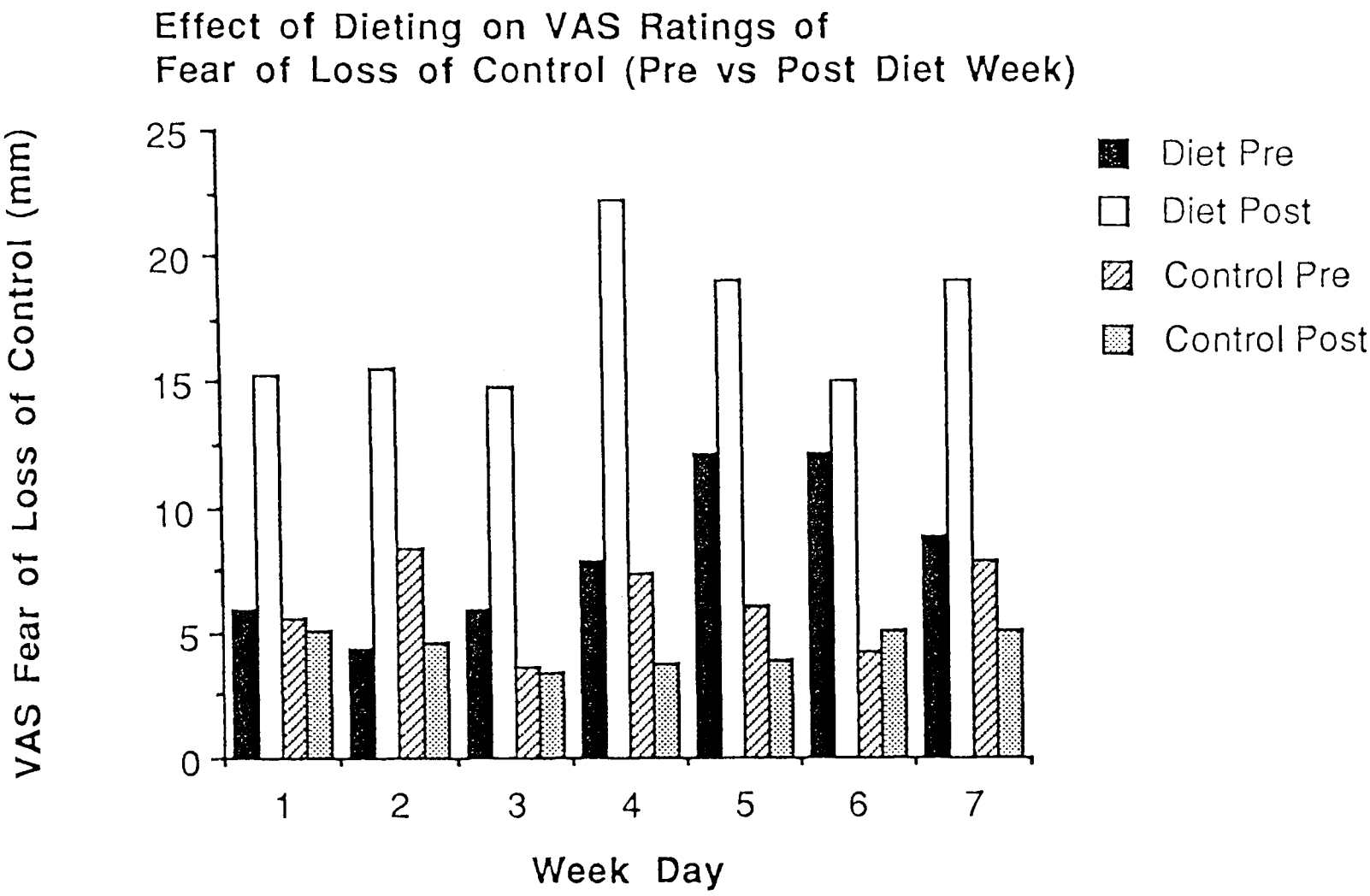

Effect of Dieting on VAS Ratings of
Fear of Loss of Control (Pre vs Post Diet Week)
VAS Fear of Loss of Control (mm)
25
20
15
10
5
0
1
2
3
4
5
6
7
Week Day
Diet Pre
Diet Post
Control Pre
Control Post

Fig 3.6

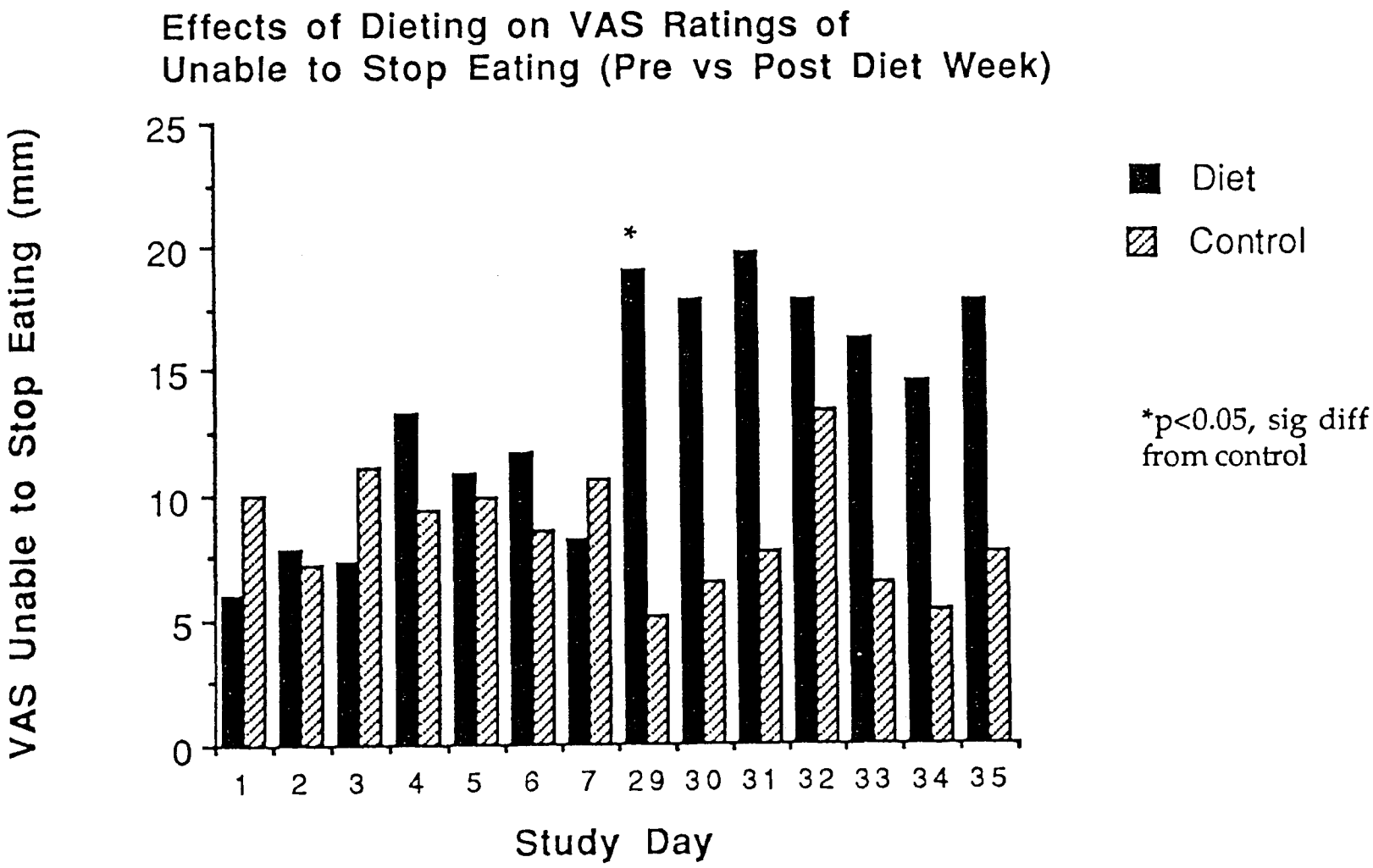

Effects of Dieting on VAS Ratings of
Unable to Stop Eating (Pre vs Post Diet Week)
VAS Unable to Stop Eating (mm)
25
20
15
10
5
0
*
1
2
3
4
5
6
7
29
30
31
32
33
34
35
Study Day
Diet
Control
*p<0.05, sig diff
from control

Fig 3.7

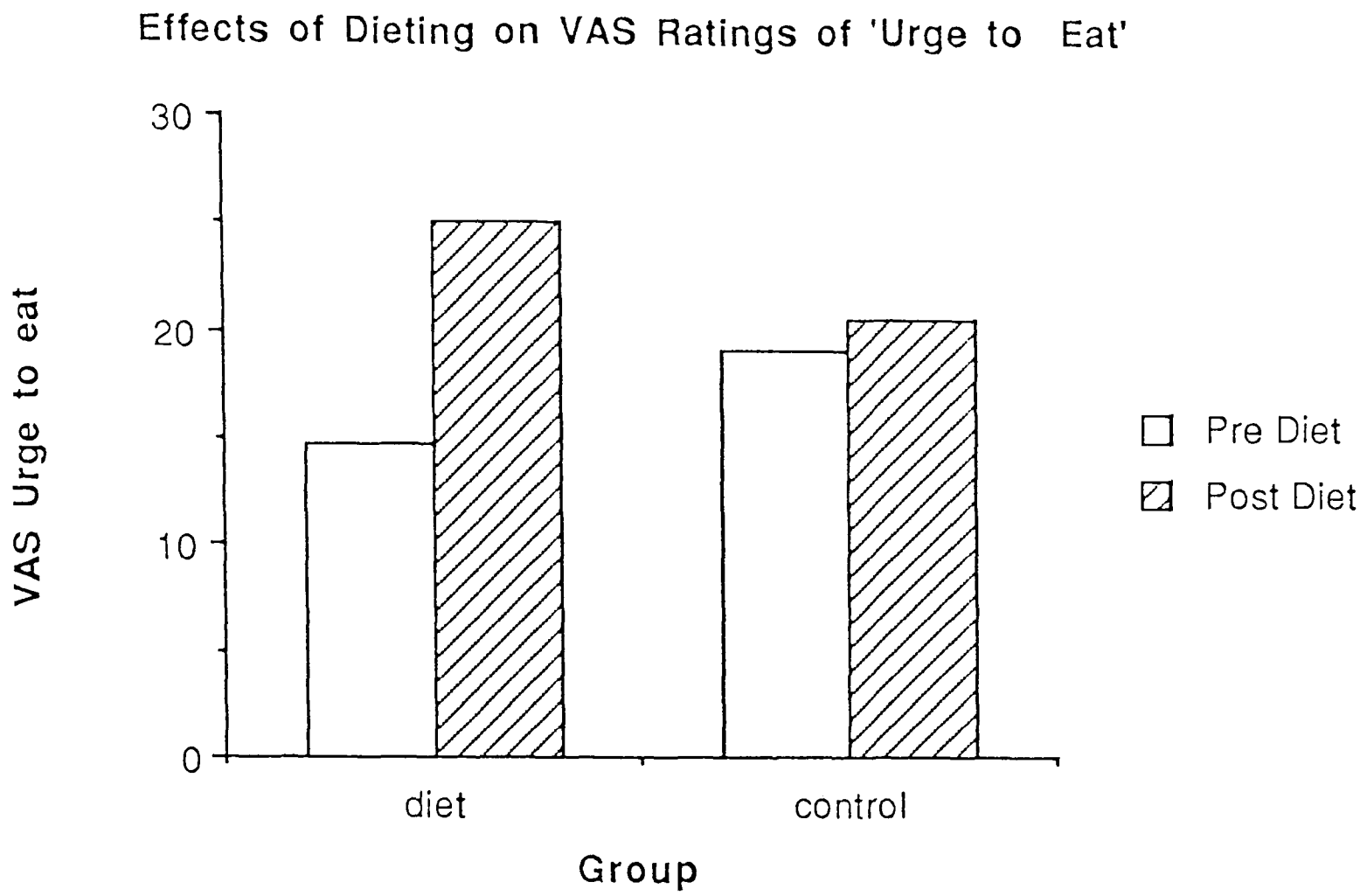
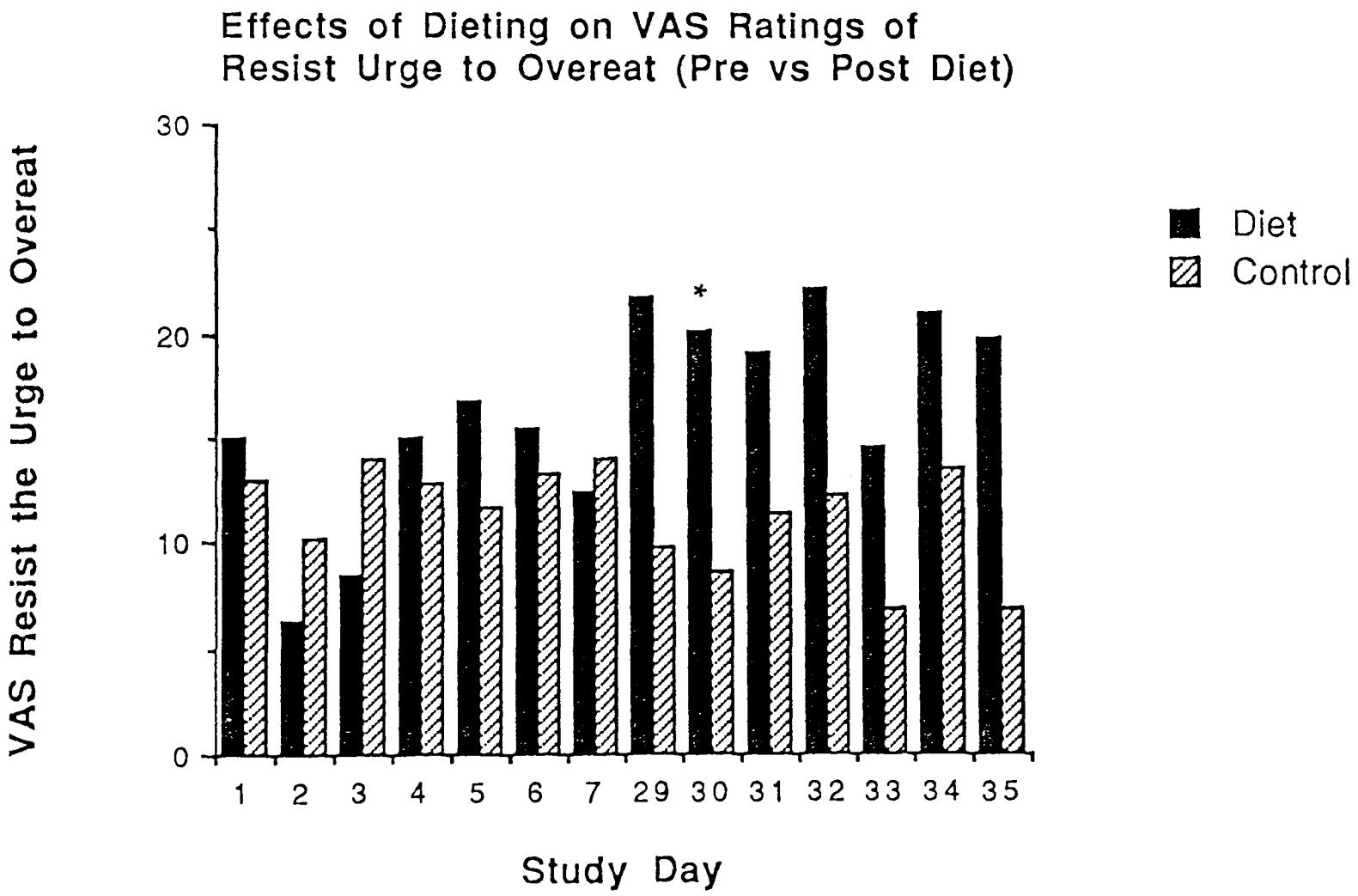

Effects of Dieting on VAS Ratings of 'Urge to Eat'
VAS Urge to eat
30
20
10
0
diet
control
Group
Pre Diet
Post Diet

Fig 3.8


Effects of Dieting on VAS Ratings of
Resist Urge to Overeat (Pre vs Post Diet)
VAS Resist the Urge to Overeat
30
20
10
0
*
1 2 3 4 5 6 7 29 30 31 32 33 34 35
Study Day
Diet
Control

Mood Measures

VAS ratings of mood, POMS and BDI scores were analyzed in order to examine the effects of dieting on mood. Daily VAS ratings of mood across the 5 weeks of the study revealed no significant main effects, or group by time interactions, suggesting that dieting had no effect on mood. This finding was reflected in the BDI scores taken pre-diet, end of diet, and post-diet; here there was no significant main effect of group ($F_{1,19}$=0.71, p>0.05), and no group by time interaction ($F_{2,40}$=0.52, p>0.05) (Tables 3.10 and 3.11).

Correlational analyses.

These were carried out to examine the association between measures of plasma tryptophan and specific behavioural measures. These were VAS ratings of hunger, despondent/sad, irritability, test meal total calorie consumption and total carbohydrate consumption, and weight.

Appetite

Ratings of hunger were expected to correlate negatively with plasma tryptophan. However, change from baseline week of VAS ratings of hunger did not correlate significantly with change from baseline plasma total tryptophan for either group (Dieters:
r=0.09, -0.05, 0.21, respectively; Controls:
r=0.17, 0.05, 0.44, respectively for the three dieting weeks; all p values >0.05). This pattern held when both groups were pooled (r=-0.12, -0.03, 0.16, respectively; all p values >0.05).

For analysis of test meal, it was expected that total calorie consumption and total carbohydrate consumption would correlate negatively with plasma tryptophan. Change in calorie consumption was therefore correlated with change in plasma total tryptophan. Thus, for calorie consumption, second test meal values were subtracted from the first; tryptophan values were derived from samples taken two days after each test meal.

Change in total calorie intake did not correlate significantly with change in plasma total or free tryptophan ($r=0.25$, $p>0.05$; $r=-0.11$, $p>0.05$, respectively). This was also true for total carbohydrate intake ($r=0.21$, $p>0.05$; $r=-0.06$, $p>0.05$).

Mood

Correlational analyses were carried out in order to examine the assumption that changes in mood ratings would be positively correlated with change in tryptophan. Change from baseline was studied at weekly intervals. For VAS ratings of despondent/sad, there were no significant correlations with change from baseline plasma total tryptophan for either group (Dieters: $r=0.01$, 0.24, -0.33; Controls: $r=0.50$, -0.02, 0.49, respectively; all p values >0.05), or for the pooled groups $r=0.23$, 0.02, -0.18, respectively; all p values >0.05).

Similarly, for ratings of irritability where there was no significant correlation of VAS ratings and change in plasma total tryptophan for dieters (dieters: $r=0.04$, 0.01, -0.37, respectively; all p values >0.05). However, for control dieters, during the first dieting week equivalent there was a significant positive correlation between change from baseline scores ($r=0.64$, $p<0.01$). This association did not hold for the remaining weeks (controls: $r=-0.39$, -0.41, respectively; both p values >0.05).

Weight

For correlation of change in weight with change in total tryptophan, there was no significant correlation for the pooled groups across the pre-diet week ($r=-0.29$, $p>0.05$). There were no significant correlation for the three diet weeks for both dieters and controls ($r=-0.46$, 0.16, 0.36, for diet weeks 1 to 3 respectively; all p values >0.05), (Controls $r=0.18$, -0.34, 0.29 for weeks 1 to 3 respectively; all p values >0.05).

1.4 DISCUSSION

Biochemical Measures. This study further elucidates the alterations in plasma tryptophan seen in individuals who comply with a 1000 kcal weight-reducing diet. The data demonstrate that the reduction in total plasma tryptophan seen with dieting is most marked after the initial diet week, with this decrease reaching a plateau for the remaining two weeks. Analysis of a control group suggested that this alteration in plasma tryptophan was in response to the diet and weight loss rather than an effect of time.

Appetite. This study further demonstrated alterations in measures of appetite and eating behaviour in response to dieting. Surprisingly the dieters did not report an increase in VAS ratings of hunger, but VAS ratings of eating disturbance revealed interesting group differences. Despite the lack of a group by time interactions for pre- and post- diet comparisons of 'fear of loss of control' and 'feeling unable to stop eating once started', the dieters appeared to experience an increase in ratings on both these measures post-dieting (Fig 3.5 and 3.7). However, this effect was confounded by an increase in ratings at the end of the pre-diet week for the dieters. It is possible that these increases were a result of the volunteers' awareness of their group allocation. Volunteers were kept blind for as long as possible, but it proved impossible to keep them blind for the last 2-3 days of the pre-diet week, as dieters only were invited to take part in a neuroendocrine challenge test (data reported elsewhere), and participants were therefore informed of group allocation after their test meal. The data would therefore suggest that simply having the intent to restrain eating may alter an individuals perceptions and experiences of food and eating. Such a phenomena would be an example of how psychological factors may affect the development of abnormal eating behaviour independently of biological factors. These alterations in VAS

ratings of eating disturbance were also seen as trends for' having strong urges to eat', and 'resisting the urge to overeat' which suggests that such alterations may occur in response to several different aspects of appetite and regulation of food intake. In order to understand this more fully, further research should concentrate on the impact of knowledge of a period of impending restraint on eating behaviour and attitudes to food. This may be important in understanding the development and maintenance of eating disorders such as bulimia nervosa where individuals are often planning to restrain their eating in response to episodes or periods of unrestrained eating.

Interestingly, these group differences in self-report ratings were not supported by interview assessment of actual episodes of disturbed eating (assessed using the EDE-Q3). Furthermore, dieting did not have an impact on this objective measure of eating disturbance. This held true for all types of eating disturbance which does not support the hypothesis that dieting can exacerbate episodes of overeating (Polivy and Herman, 1985). However, it remains of interest to speculate whether a longer or more strenuous dieting regime would have exacerbated these trends for increases in ratings of eating disturbance, and whether this may have been associated with more objective changes in eating behaviour.

The more direct assessment of eating using the test meal protocol revealed no changes in amount eaten or macronutrient choice, or in VAS ratings of satiety pre- and post-meal. Thus, counter to hypothesis, dieting was not associated with increased calorie consumption and increased carbohydrate consumption. There was, however, a trend for dieters to have an increased preference for carbohydrate items on the food preference questionnaire after dieting compared with controls. Unfortunately this trend contrasts with the forced choice questionnaire which suggested that dieters had a trend to select fewer carbohydrate items

than controls (although this did not interact with time) when forced to choose between carbohydrate and protein. Interpretation of the data is complicated by the possibility that dieters found it difficult to break their dietary rules and eat freely in a laboratory setting. It may have been more appropriate to carry out the second test meal at the moment the diet ended rather than expecting dieters to temporarily break their diet rules. Unfortunately the former option was not possible with this study, as data would have been confounded by the neuroendocrine challenge test.

Mood. The data did not suggest that dieting had a negative impact on mood. This finding is in agreement with Pirke et al (1985) and Warren and Cooper (1988), two studies of moderate diet regimes. It would appear that this affect is seen mainly in the severe, semi-starvation conditions (Keys et al, 1950; Fichter et al, 1985). Thus this study does not suggest that moderate dieting may be associated with a decrease in mood which will further predispose an individual to develop an eating disorder and related mood disorder. It would appear that a very stringent self-imposed regime of food restriction would be necessary to bring about changes in mood in such an individual, suggesting that this may occur during the more advanced stages of the illness.

Interestingly, and counter to prediction, there appeared to be no association between alterations in plasma tryptophan and ratings of mood or appetite as examined with correlational analyses. Such an association would have provided support for the hypothesis that alterations in tryptophan have an impact on behaviour. The reductions in plasma tryptophan were not directly correlated with weight loss, suggesting that intervening variables mediate the effect of weight loss on plasma tryptophan.

In conclusion, surprisingly few effects on behaviour were observed in this study. These findings suggest that healthy females are not vulnerable to the effects of dieting on behaviour and mood. However, volunteers for this study underwent a detailed screening procedure, and were therefore not a representative sample of the female population. For example, volunteers with personal or family history of psychiatric disorder were excluded. It has been argued that it is precisely this group of women who are at risk of developing clinical eating disorders in that there is increased prevalence in individuals with first degree relatives with affective disorder (Hudson et al, 1983b; Kassett et al, 1989). This would then suggest that in healthy females, dieting behaviour is not hazardous. It would therefore be interesting to compare responses of sub-groups of dieters with different risk factors for developing eating disorders or depression.

The role of alterations in plasma tryptophan on behaviour remains unclear. This study did not reveal associations, but does not allow a conclusion of no association because of the confounding effects of the diet. In order to pursue more directly the relationship between tryptophan depletion and changes in mood and appetite, it would be necessary to manipulate plasma tryptophan independently of dieting, and to carry this out in a controlled laboratory setting. This and the question of vulnerability to tryptophan depletion will be addressed in subsequent studies.

CHAPTER 4 THE EFFECT OF ACUTE TRYPTOPHAN DEPLETION ON MOOD AND APPETITE IN HEALTHY FEMALE VOLUNTEERS

4.1 INTRODUCTION

The previous study demonstrated that dieting significantly lowers plasma tryptophan. Decreased availability of tryptophan to the brain will decrease the synthesis of 5-HT (Biggio et al, 1974; Moja et al, 1989) (section 1.1.2). The evidence that 5-HT is involved in the regulation of appetite and mood is reviewed in sections 1.5.2.3 and 1.2. It is therefore important to consider the effects of tryptophan reduction on human behaviour. The study of dieting did not allow for a clear distinction between the effects of dieting behaviour and tryptophan reduction, and it is therefore important to consider the effects of tryptophan depletion in a controlled laboratory setting.

Whilst the relationship between increased 5-HT and lowered appetite has been well documented (section 1.5.3.2), the relationship between increased appetite and decreased 5-HT function has been less well established. There is evidence to suggest that in certain experimental settings, a reduction of 5-HT function is associated with an increase of food intake (Weiss et al, 1986; Stallone and Nicolaidis, 1989). This phenomenon is less robust, partly because of the limitations of the methodologies available for reducing 5-HT synthesis or neurotransmission. However, one study using a very similar protocol to that adopted for this study reports an alteration in appetite characterised by a small decrease in protein intake (in male volunteers) (Young et al, 1988). This finding does not follow the alteration predicted by the hypothesis that lowering brain 5-HT would result in an increased caloric consumption, with an increase in carbohydrate foods compared with other macronutrients.

However, the study was limited by the method of appetite assessment; whilst volunteers were given a free feeding buffet style meal, the foods available to them were limited, and did not offer a wide enough choice in order to interpret the finding of decreased protein consumption.

Evidence suggests that tryptophan depletion is associated with decrease in mood in both depressed patients maintained on antidepressant medication, and healthy volunteers. Acute tryptophan reduction is associated with a rapid return of symptoms in remitted depressed patients (Delgado et al, 1990). Whilst it seems likely that the explanation for this involves a reversal of the antidepressant medication, reports of subtle alterations in mood in healthy volunteers suggest that simply reducing tryptophan availability will affect normal cognitive functioning (Young et al, 1985; Smith et al, 1987).

These effects, however, have not been examined in healthy female populations. Given the increased incidence of depressive disorder and eating disorders amongst females, it is of particular interest to examine the effect of tryptophan depletion in this group. Previous studies indicate that females may be more vulnerable than males to the effects of dieting-induced tryptophan depletion (Anderson et al, 1990b). Section 1.5.2.3 further reviews the evidence for sexual dimorphism in relation to 5-HT function and appetite.

The purpose of the present study was, therefore, to study the effects of acute tryptophan depletion on mood, appetite and food intake in a female population. It was hypothesised that tryptophan depletion would significantly lower mood. A further prediction was that tryptophan depletion would increase caloric consumption, which would be characterised by an increase in carbohydrate foods.

4.2 METHODS

Volunteers

12 healthy female volunteers were recruited through advertisement to take part in a study to look at the effects of diet and food intake on mood. All volunteers were carefully screened to ensure that they were in good health, had no history of psychiatric disorder or eating difficulties, and no current dietary restriction or eating difficulties (two subjects were non-strict vegetarians). Assessment of physical health and psychiatric history were carried out by a psychiatrist specialising in this area. Volunteers were required to fall within the normal range of weight for height (BMI), ie 17-30, and to score within the normal range for current attitudes to eating as scored by the Eating Attitudes Test (EAT) (Garner and Garfinkel, 1979), ie 0-20. The Beck Depression Inventory (BDI) (Beck et al, 1961) was used to assess current ratings of depression, and an upper limit of 20 was placed on this for inclusion into the study.

Volunteers were between the ages of 19 and 27 (mean=22.3, SD±2.8), and of normal weight for height (BMI range 17.4-29.0, mean=23.0, SD±3.4). They all fell within the normal range for depression (BDI) (range 0-17, mean=6.4, SD±6.2). Volunteers fell within the normal range on the EAT questionnaire (range 2-20, mean=9.6, SD±7.0), and had a regular 28 day menstrual cycle.

Experimental Design

The experiment was a within subjects comparison where the tryptophan depletion condition

(T-) was compared with a balanced amino acid condition (B), and a placebo control condition. Thus subjects were required to attend the research unit for three experimental sessions in order to complete the study; these were a minimum of one week apart. These sessions were carried out between days 3 and 21 of the menstrual cycle in order to avoid the late luteal phase of the cycle. In order to do this, it was necessary for most volunteers to carry out the three experimental sessions over two consecutive menstrual cycles. It was ensured that no systematic bias was employed in the allocation of these sessions to a particular phase of the cycle.

Because of the highly distinctive consistency and taste of the two amino acid mixtures, it was necessary to adopt a semi-balanced design with the placebo amino acid-free condition always given first in a single blind fashion. Thus the remaining two conditions remained double blind and were administered in a cross-over fashion. Order effects were therefore gauged on the basis of the T- and B drinks, with the placebo condition being used as an anchor for the group comparison. The design is set out below.

DESIGN OF TRYPTOPHAN DEPLETION STUDY			
GROUP SIZE	SESSION 1	SESSION 2	SESSION 3
6	Placebo	(B) Mixture	(T-) Mixture
6	Placebo	(T-) Mixture	(B) Mixture

Dietary Manipulation

Plasma tryptophan was lowered using the 50g amino acid loading technique outlined in the general methods chapter (section 2.1). This section also contains an outline of the control balanced drink, and the placebo drink.

Measures

Mood. This was measured using the Profile of Moods State Questionnaire (POMS) (McNair et al, 1971) which had been amended to examine current mood, and 100mm visual analogue scales (VAS). (Appendix 2.10). For VAS, the parameters were despondent/sad, anxious, irritable, and tense. These measures were also given to subjects to take home and complete for the remainder of the day and for the next day.

Appetite. This was measured using VAS ratings of hunger across the test morning. Appetite was also assessed using the standardised test meal procedure set out in section 2.2. This included the standard buffet style free selection test meal with pre- and post-meal rating scales (see section 2.2 for methodology). In addition to these scales, VAS measures of disordered eating were included (Appendix 4.1). These were included to enable comparison in a subsequent study with a group of women who had recovered from an eating disorder (Chapter 5). These data were not analyzed for the present study.

All measures required subjects to rate how they felt at that moment.

Procedure

Each experimental session was identical with the exception of the drink manipulation. Volunteers were asked to eat normally during the day preceding the test session, and to fast from midnight of the night before. They were asked to drink nothing other than water. The timetable for the session is set out below.

TRYPTOPHAN DEPLETION STUDY PROTOCOL	
TIME	MEASURE
08.30	Subject arrive. Settle in test room
08.40	Baseline VAS (mood and hunger), POMS, and Food Preference Questionnaire
08.50	10ml baseline fasting plasma sample taken
09.00	Drink administered
09.30-13.30	VAS (mood and hunger) at hourly intervals
13.30	VAS (mood and hunger), POMS, and pre-meal VAS
13.35	+4.5 hour 10ml plasma sample taken
13.40	Test meal followed by rating scales as for 13.30
14.15 approx	Session end. Subject sent home with rating scale booklet to complete before and after their evening meal and lunch the next day

Biochemical Measures

10ml blood samples were taken into heparin tubes prior to the drink ('0' mins) and 4.5 hours after the drink. Plasma samples were separated by centrifugation and stored at -30°C. Plasma total and free tryptophan were determined by fluorometric detection according to the method of Bloxam and Warren (1974), and Bloxam et al (1977). The methodologies for these assays are outlined in section 2.4.

Data Analysis

All data were analyzed by analysis of variance using the BMDP p2V statistical package or the ANOVA suite on Supastat (SPA). This analysis was group (order 1 vs. order 2). Greenhouse Geisser probability was used where repeat measures were part of the analysis.

4.3 RESULTS

Biochemical Measures

Plasma concentrations of total and free tryptophan were analyzed by analysis of variance for all three conditions.

For plasma total tryptophan, the ANOVA yielded a significant main effect of treatment ($F_{2,22}$ =33.77, $p<0.001$) and of time ($F_{1,11}$ =57.1, $p<0.001$). There was also a significant treatment by time interaction ($F_{2,22}$ = 45.13, $p<0.001$). Post hoc T-tests revealed that there was a highly significant fall in total tryptophan levels in the (T-) condition ($p<0.0005$). This fall constituted a 78% decrease in plasma tryptophan across the 4.5 hours. With the placebo drink condition there was also a significant fall of tryptophan levels with time ($p<0.0005$) although this was of a lesser magnitude; a 23% decrease across the 4.5 hours. There was no significant difference between baseline and +4.5 hours for the balanced drink condition (Fig 4.1).

For analysis of plasma free tryptophan, the ANOVA revealed a significant main effect of treatment ($F_{2,22}$=11.14, $p<0.001$) and of time ($F_{1,11}$=30.32, $p<0.001$). The treatment by time interaction was also significant ($F_{2,22}$=33.27, $p<0.001$). The pattern of change in free tryptophan concentrations was very similar to that seen with total tryptophan concentrations; in the (T-) condition, levels fell by 67% ($p<0.0005$) across the 4.5 hours compared with a 24% decline in the placebo condition ($p<0.001$). There was no significant difference between baseline and +4.5 hours for the balanced drink condition. For all of these analyses there was no significant difference between the baseline measures (Fig 4.2).

Fig 4.1

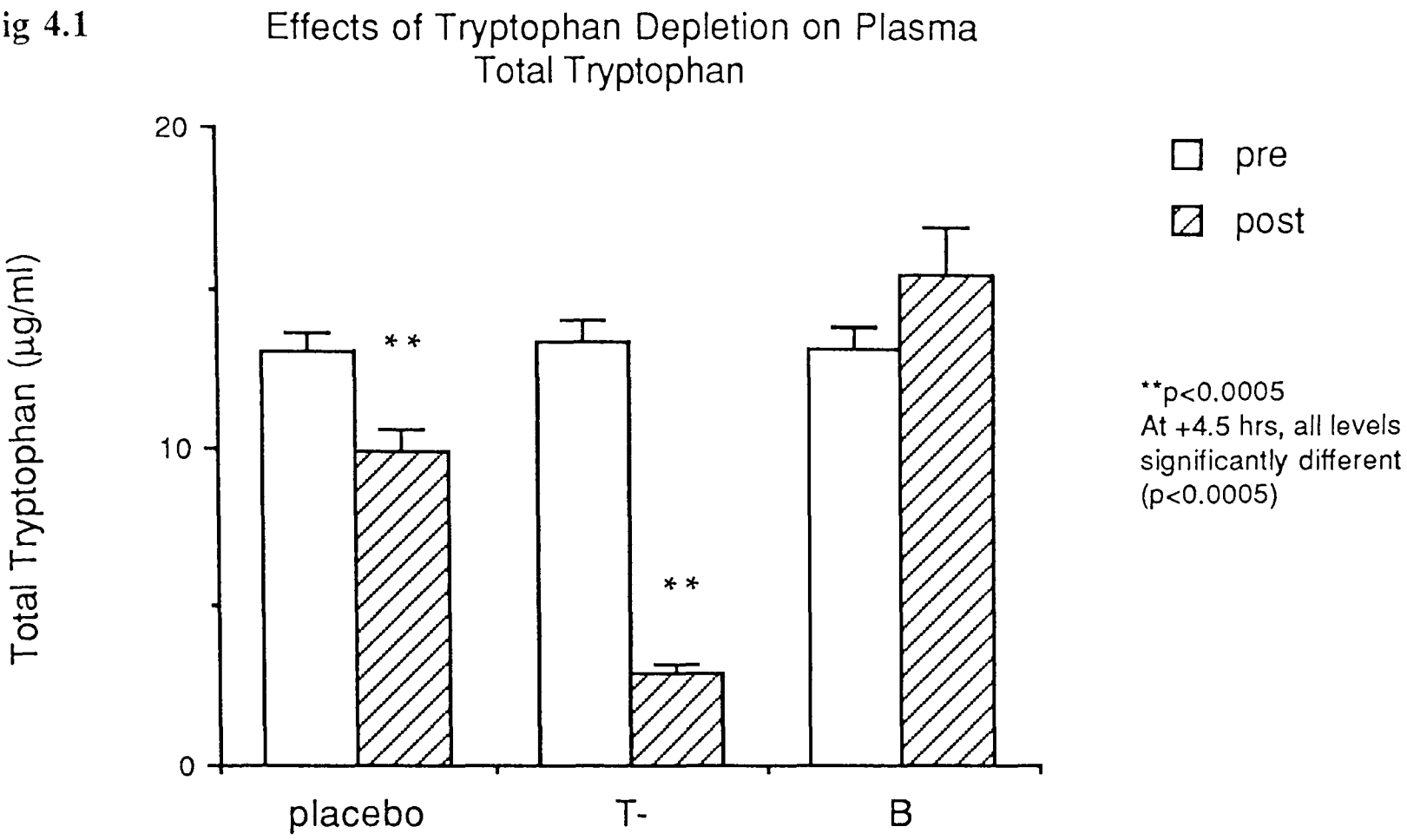
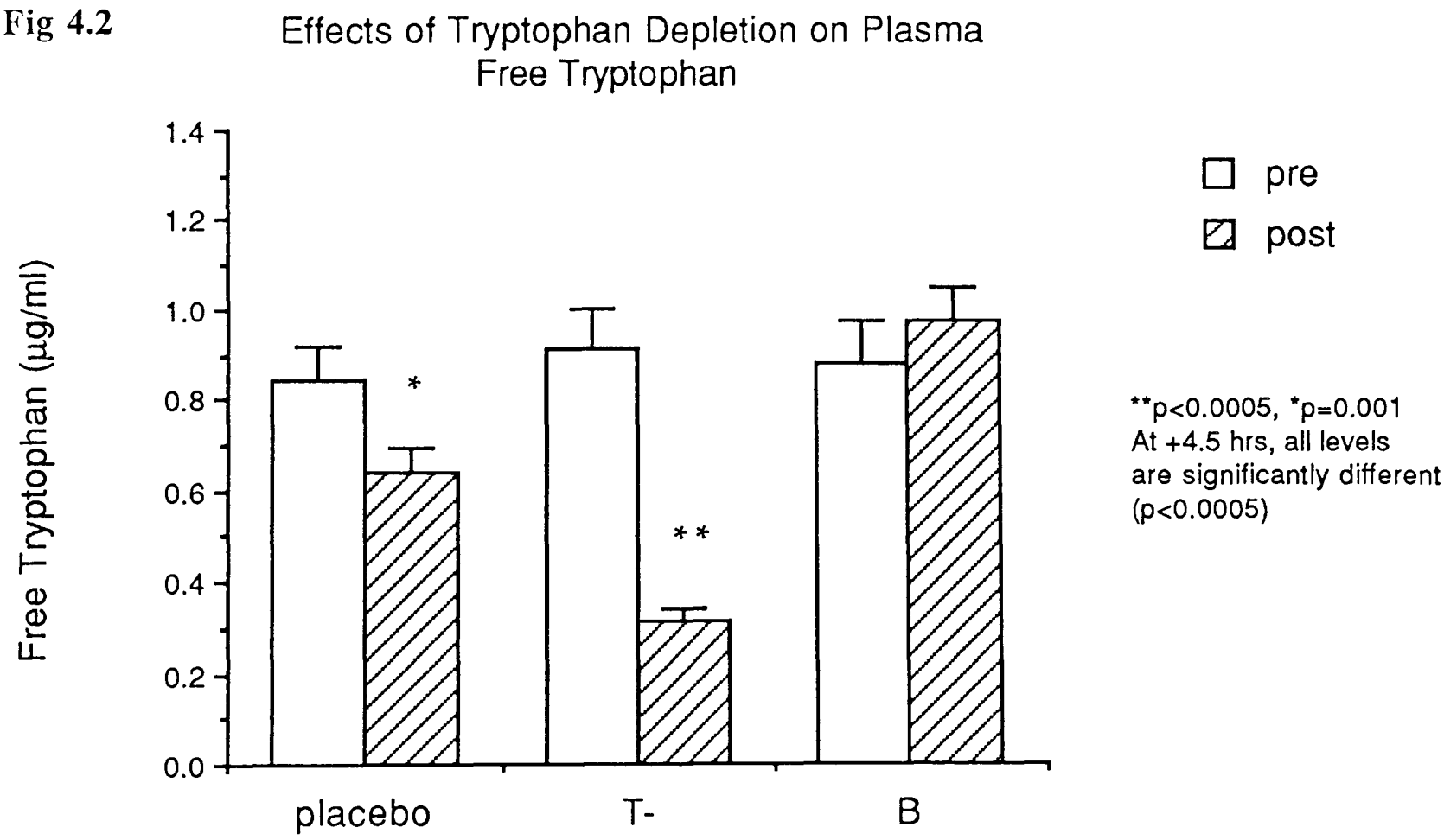

Effects of Tryptophan Depletion on Plasma
Total Tryptophan
Total Tryptophan (µg/ml)
20
10
0
**
**
placebo
T-
B
pre
post
**p<0.0005
At +4.5 hrs, all levels
significantly different
(p<0.0005)

Fig 4.2


Effects of Tryptophan Depletion on Plasma
Free Tryptophan
Free Tryptophan (µg/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
placebo
T-
B
pre
post
**p<0.0005, *p=0.001
At +4.5 hrs, all levels
are significantly different
(p<0.0005)

The means and standard errors for this data are set out in Table 4.1.

Appetite Measures.

The effects of tryptophan depletion on appetite were analyzed by ANOVA to see whether there was an increase in appetite and carbohydrate consumption as a result of tryptophan depletion. This was carried out on the test meal, food preference questionnaire and appetite related VAS's for both groups. For all of these measures, there was no effect of order in the ANOVA.

For total calories consumed at test meal there was no main effect of condition on total calorie intake (Fig 4.3). This was also true for the intake of specific macronutrients (Fig 4.4); ie for total intake of carbohydrate, protein and fat (Tables 4.2 and 4.4).

The ANOVA findings for VAS ratings of appetite were examined to look at the effects of tryptophan depletion on subjective ratings of hunger and satiety. For VAS ratings of hunger taken across the test morning (Table 4.3, Fig 4.5), there was a significant main effect of treatment, but no interaction of treatment by time. The absence of this interaction suggests that the condition effect was not an effect of the amino acid manipulation. Inspection of the means indicated a baseline difference such that the balanced drink condition ratings were considerably lower than the other two conditions, and that the condition effect was merely a contamination of this difference throughout the test session.

VAS ratings of satiety (Tables 4.3 and 4.6) were taken pre and post test meal. The parameters here were; 'wish to eat', 'feel full', and, 'find meal satisfying'. For these three

Fig 4.3

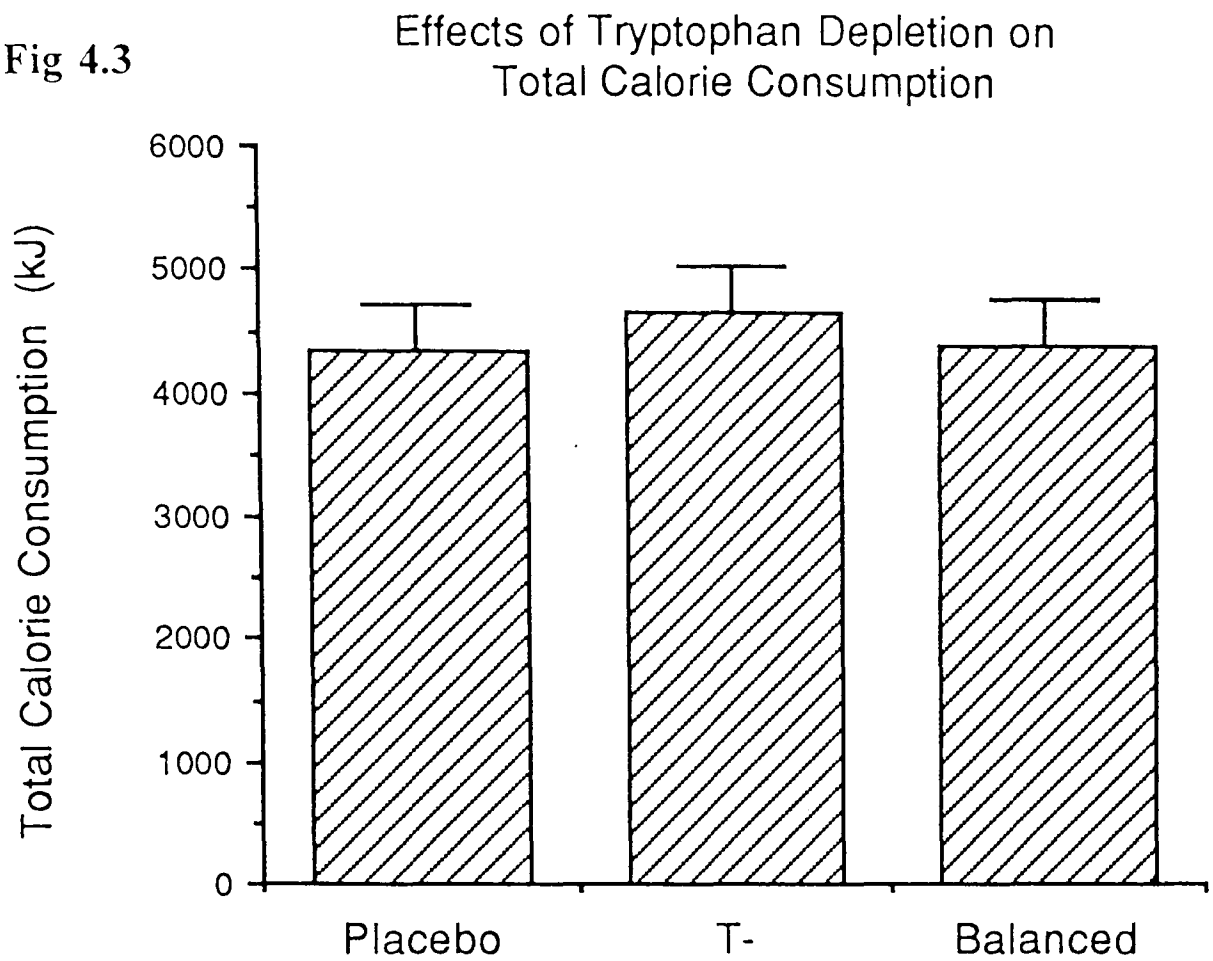

Effects of Tryptophan Depletion on
Total Calorie Consumption
Total Calorie Consumption (kJ)
6000
5000
4000
3000
2000
1000
0
Placebo
T-
Balanced

Fig 4.4

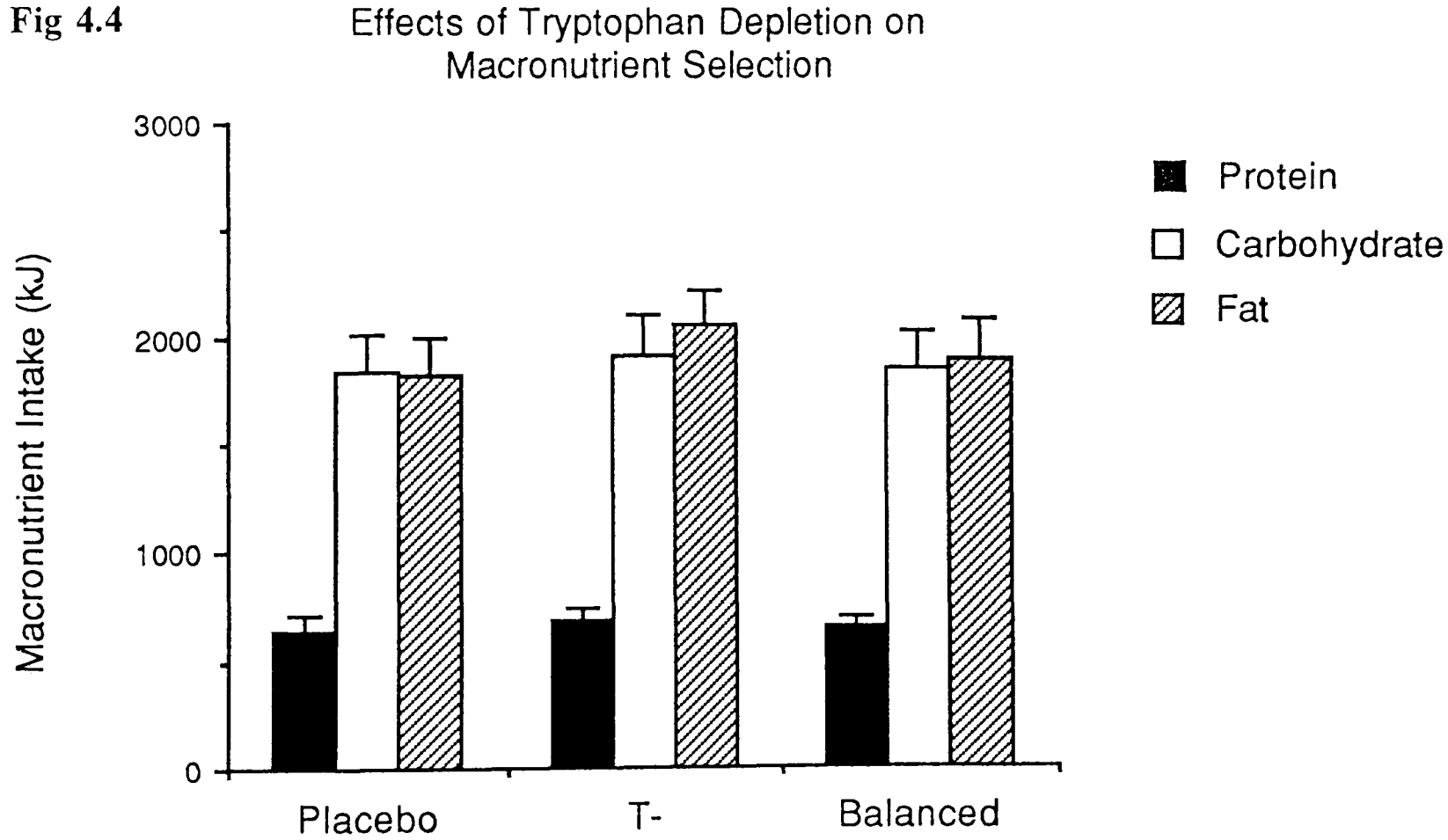
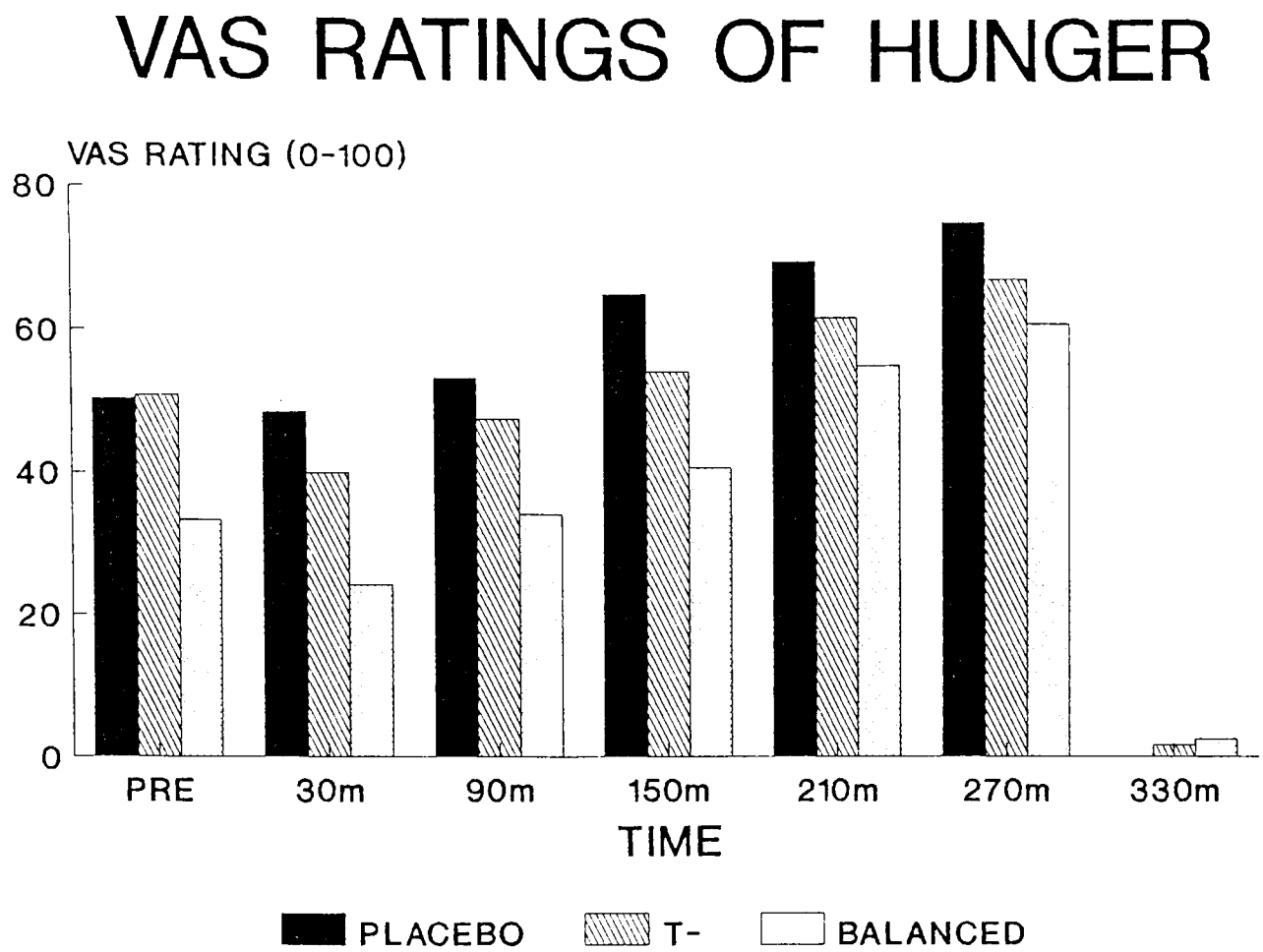

Effects of Tryptophan Depletion on
Macronutrient Selection
Macronutrient Intake (kJ)
3000
2000
1000
0
Placebo
T-
Balanced
Protein
Carbohydrate
Fat

Fig 4.5


VAS RATINGS OF HUNGER
VAS RATING (0-100)
80
60
40
20
0
PRE
30m
90m
150m
210m
270m
330m
TIME
PLACEBO
T-
BALANCED

measures there was no significant effect of condition. There was also no significant interaction of condition by time, but for 'wish to eat', there was a trend towards significance in this interaction (Tables 4.3 and 4.6). Examination of the means suggested that this effect was coming from the placebo condition where 'desire to eat' was greater than the amino acid conditions pre-meal, and relatively smaller post-meal.

Food preference questionnaires which had been given baseline and pre- and post-meal generally confirmed the findings of the objective test meal measure on macronutrient selection, ie that there was a lack of specificity of macronutrient selection between the three conditions (Tables 4.3 and 4.5). For free choice of carbohydrate (Fig 4.6) and protein (Fig 4.7), there were no significant main effects of condition, or condition by time interactions. This was also true for forced choice of carbohydrate vs protein (Fig 4.9). For free choice of fat items however, there was a significant main effect of condition, but no condition by time interaction (Fig 4.8). Inspection of the means suggested that this was a result of a decrease in fat selection on this measure pre-meal in balanced drink conditions relative to the T- and placebo condition, but this group also had a lower baseline rating. The absence of an interaction suggests that this finding was not specific to the manipulation (Table 4.3 and 4.5).

VAS ratings of disordered eating were not analyzed here as these measures were included only for comparison with an eating disordered population (Chapter 5).

Fig 4.6

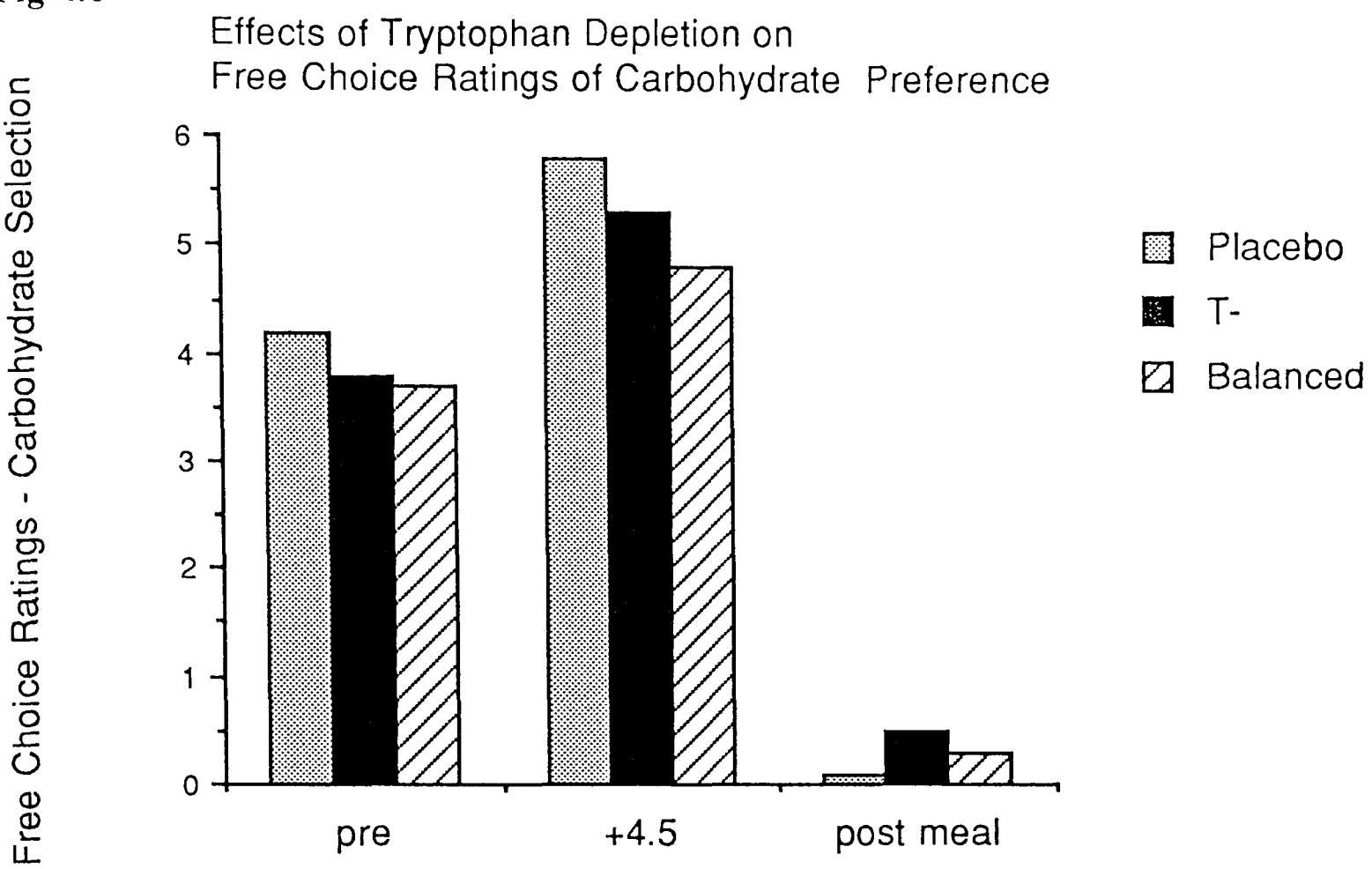

Effects of Tryptophan Depletion on
Free Choice Ratings of Carbohydrate Preference
Free Choice Ratings - Carbohydrate Selection
6
5
4
3
2
1
0
pre
+4.5
post meal
Placebo
T-
Balanced

Fig 4.7

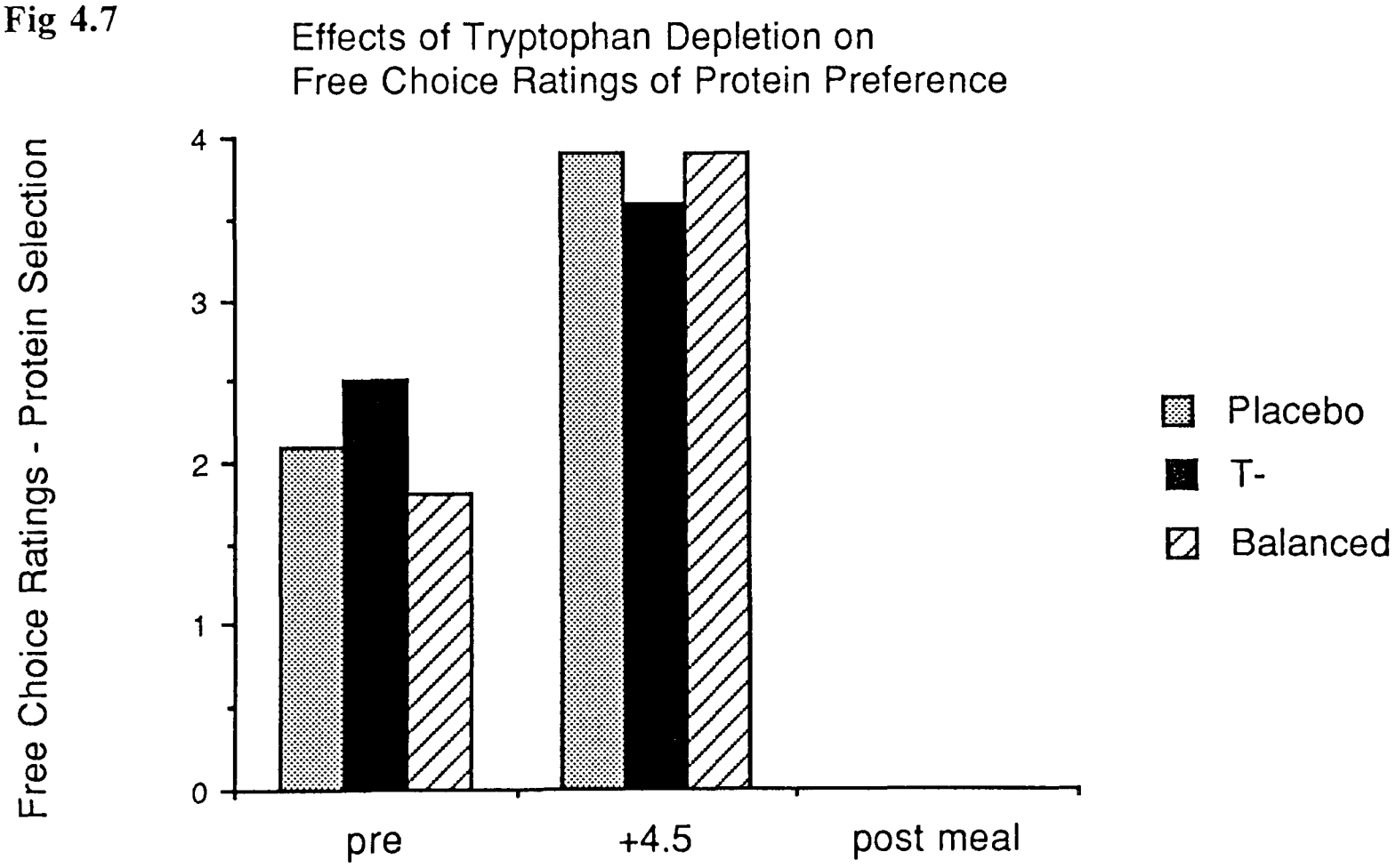

Effects of Tryptophan Depletion on
Free Choice Ratings of Protein Preference
Free Choice Ratings - Protein Selection
4
3
2
1
0
pre
+4.5
post meal
Placebo
T-
Balanced

Fig 4.8

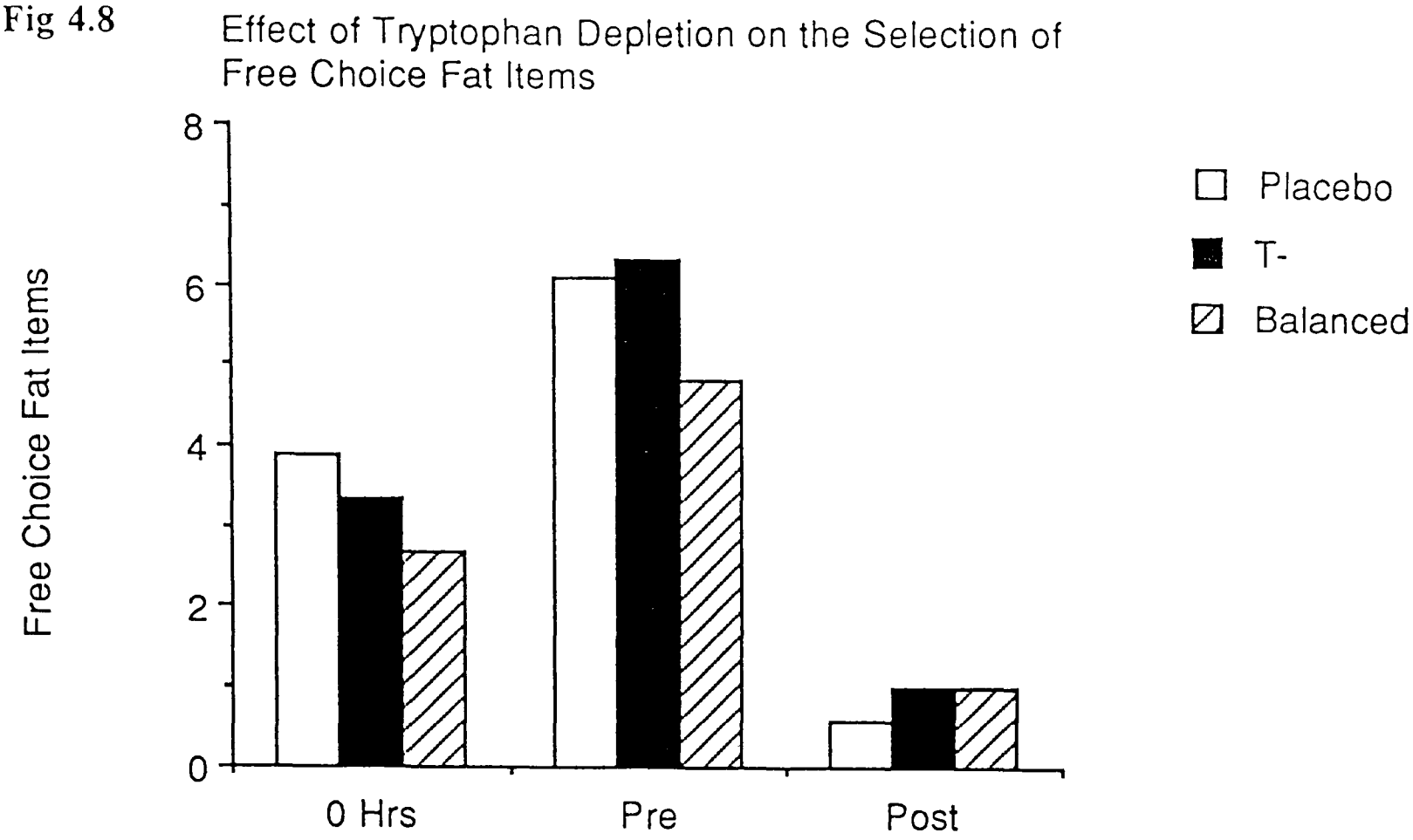
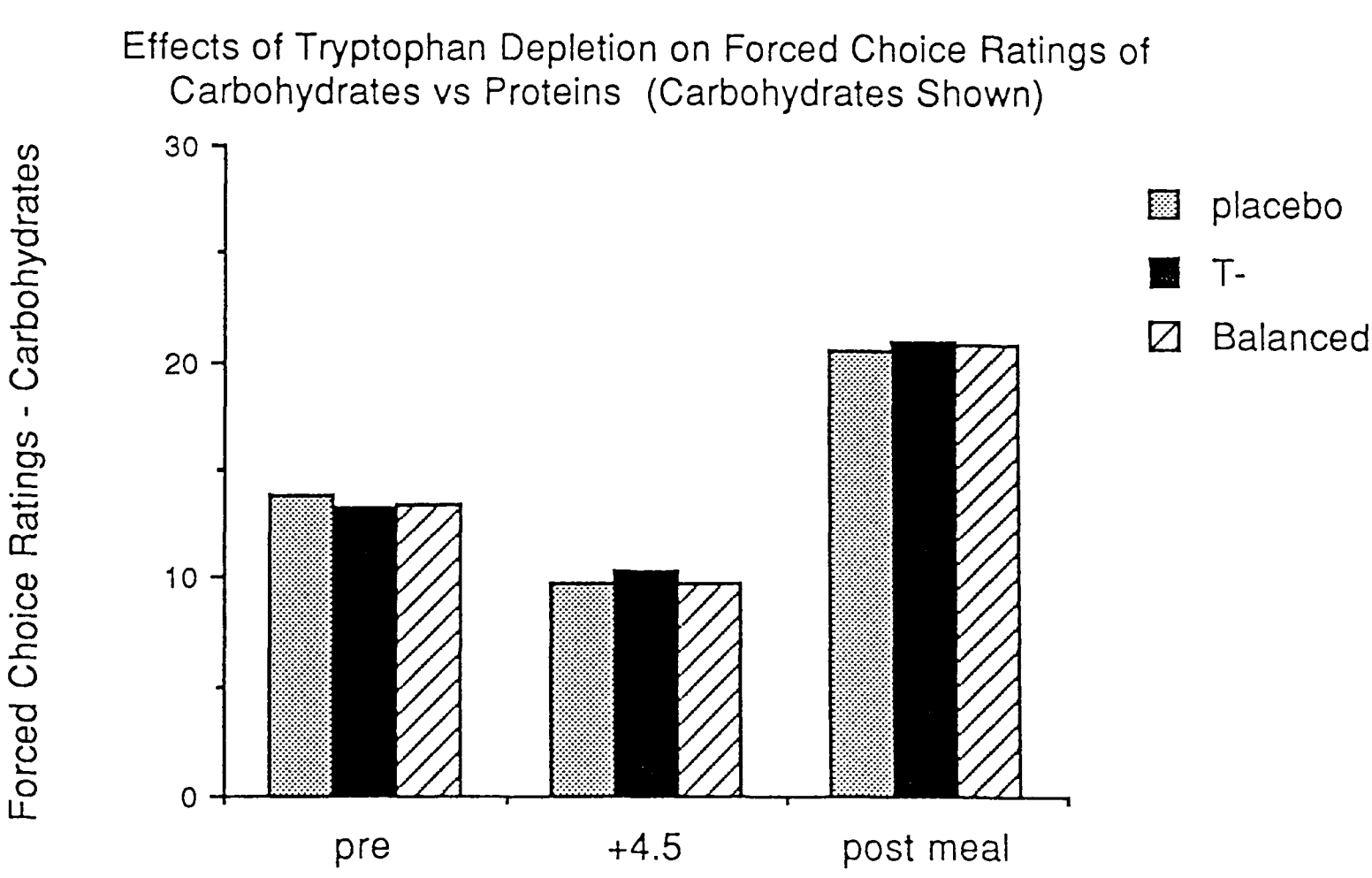

Effect of Tryptophan Depletion on the Selection of Free Choice Fat Items
Free Choice Fat Items
8
6
4
2
0
0 Hrs
Pre
Post
Placebo
T-
Balanced

Fig 4.9


Effects of Tryptophan Depletion on Forced Choice Ratings of Carbohydrates vs Proteins (Carbohydrates Shown)
Forced Choice Ratings - Carbohydrates
30
20
10
0
pre
+4.5
post meal
placebo
T-
Balanced

Mood Measures

An ANOVA was carried out on measures of mood to examine the effects of tryptophan depletion on the lowering of mood. These were VAS ratings of sadness/despondency, irritability, tension and anxiety, and POMS ratings of depression and anxiety/tension. In all of these analyses the order effect did not approach significance.

For VAS ratings of mood (Tables 4.7 and 4.9) the ANOVA revealed a significant main effect of condition for ratings of tension, anxiety, and irritability. Only for tension and anxiety was there a significant treatment by time interaction (Fig 4.10 and 4.11). However, further examination of these data revealed that all of these effects were a reflection of increased scores in the initial placebo session thus suggesting that the amino acid load per se had no impact on these scales. For VAS ratings of despondency (Fig 4.12) there were no significant main effects of condition, or condition by time interaction.

Analysis of the POMS (Table 4.7 and 4.8) ratings of depression and anxiety/tension revealed no significant main effect of condition (Fig 4.14). However, for anxiety/tension there was a trend for a condition by time interaction which just failed to reach significance (Tables 4.7 and 4.9). This interaction appeared to be coming from the initial placebo session where subjects rated themselves as more anxious at baseline and pre-meal than in the amino acid conditions, but this difference had disappeared by the end of the meal. Thus this interaction does not indicate that there was a specific effect of amino acid loading on this measure.

Fig 4.10

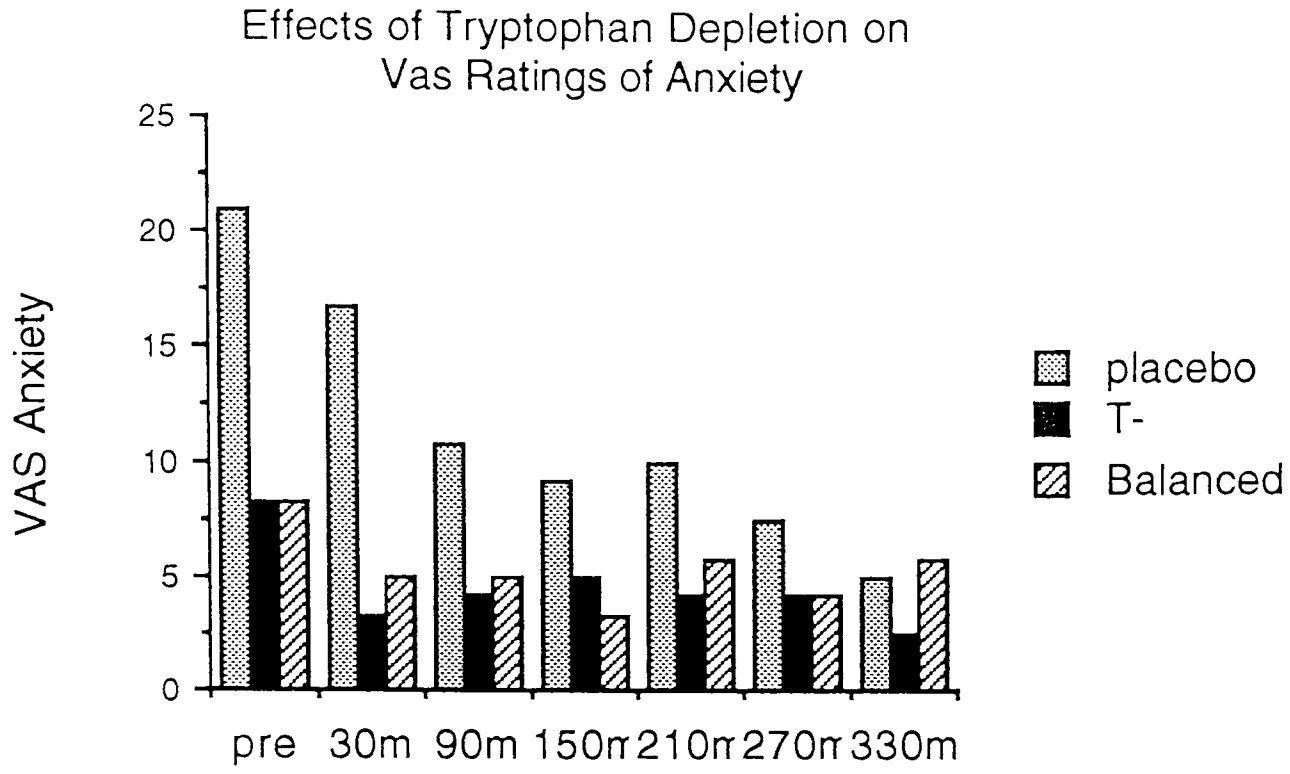


Fig 4.11

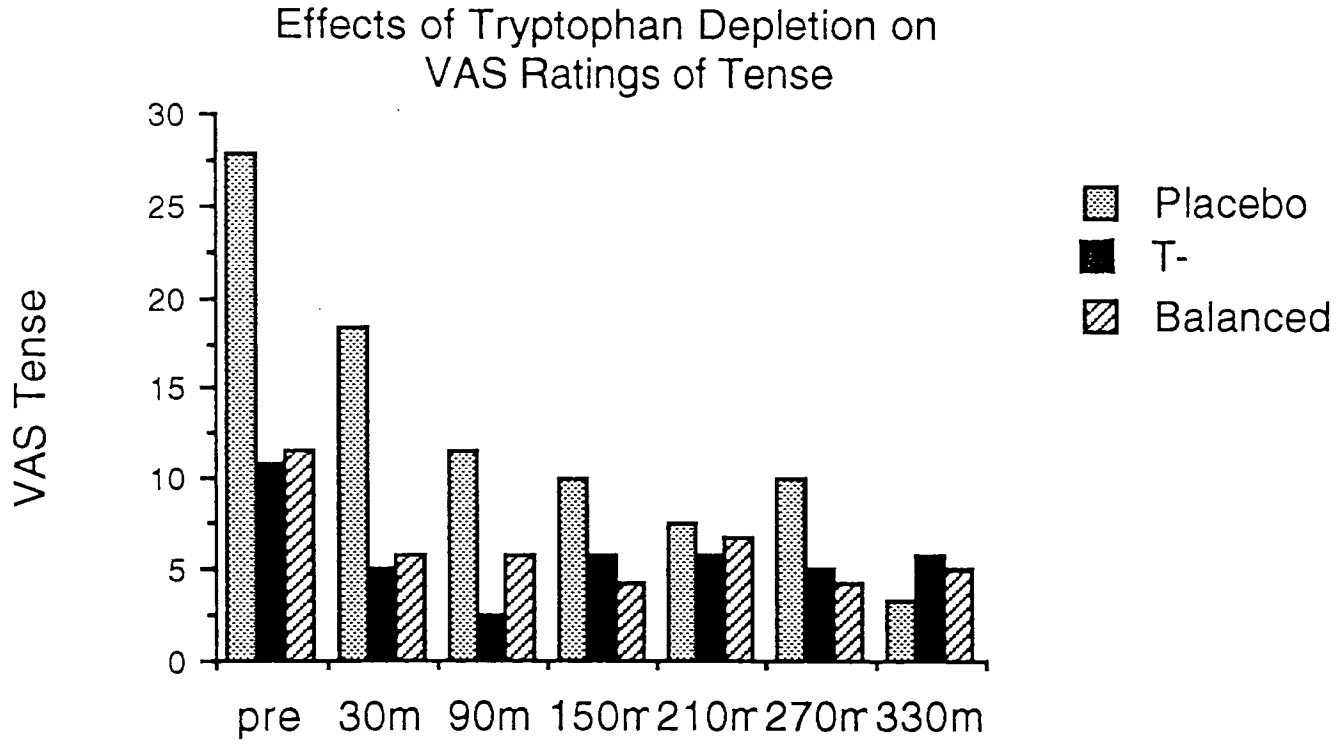


Fig 4.12

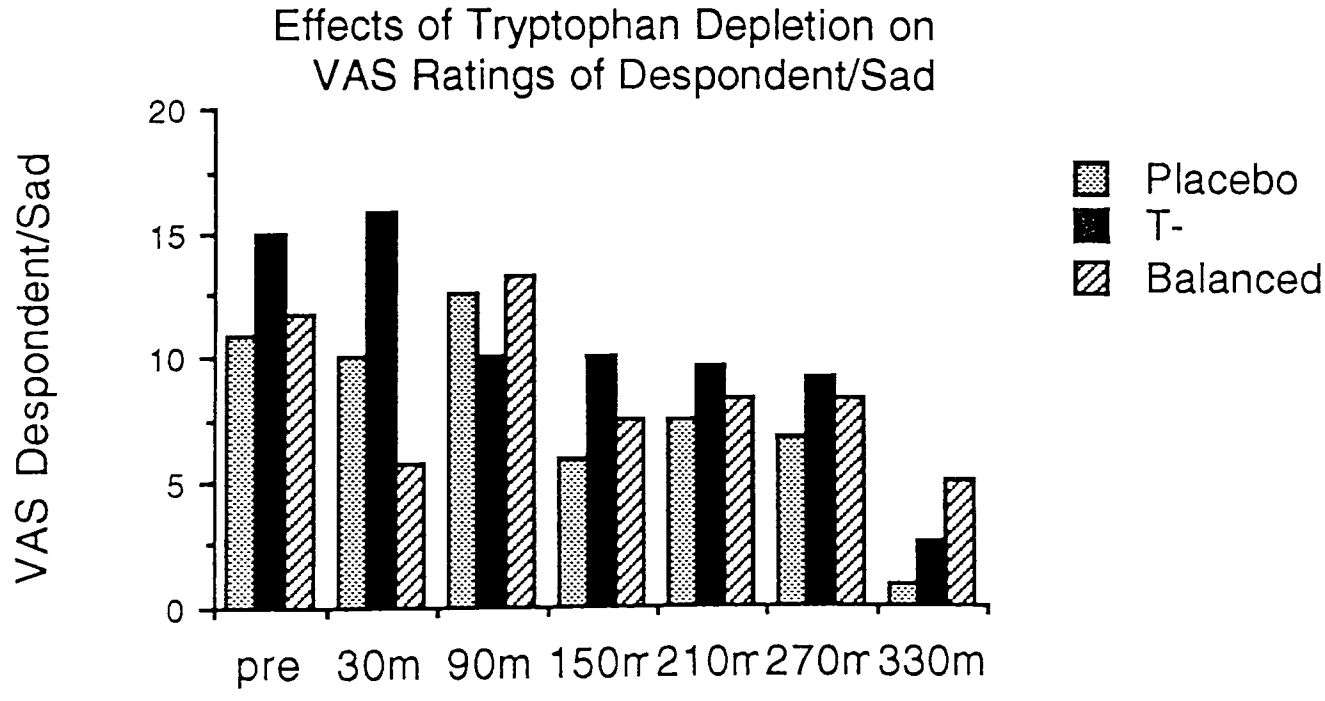


Fig 4.13

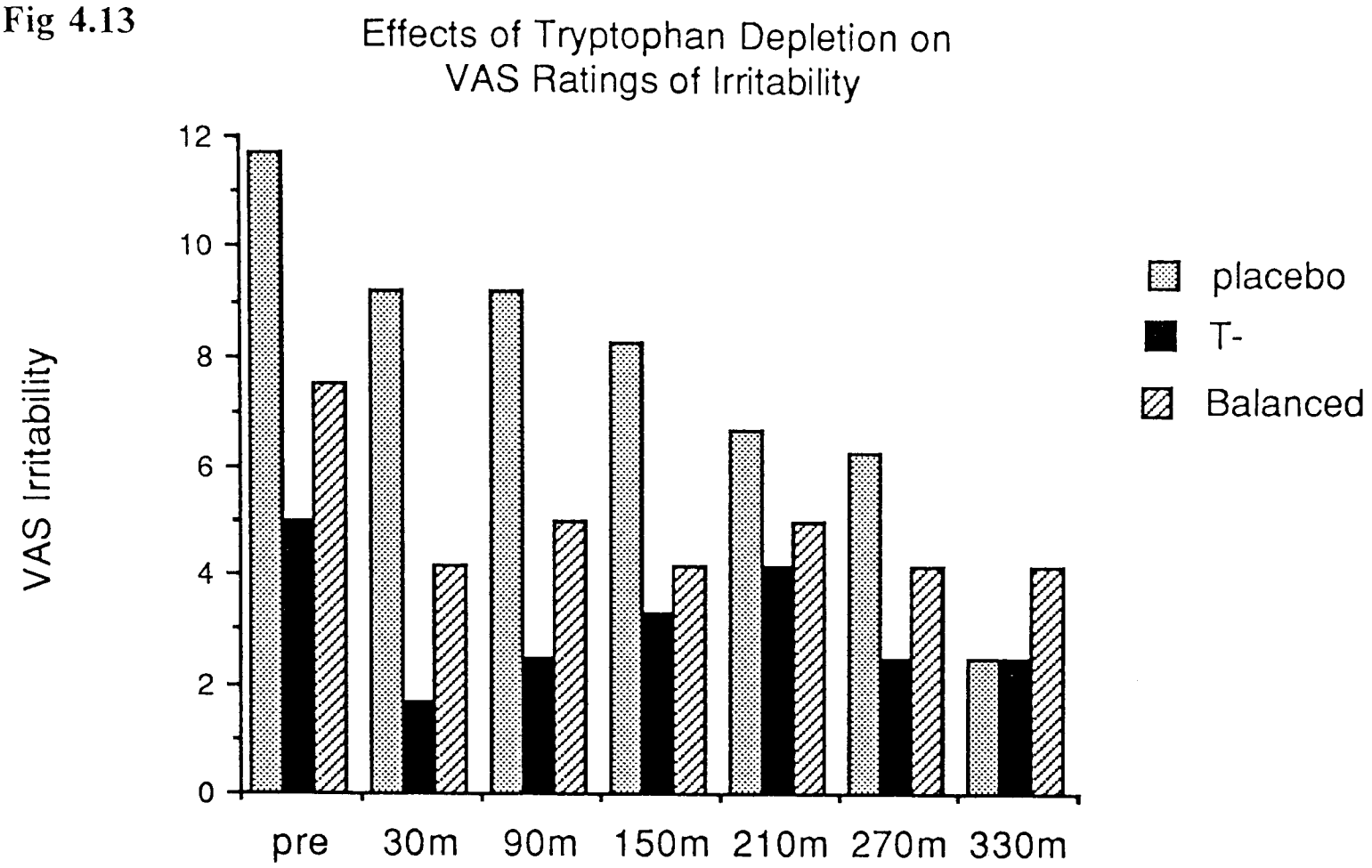
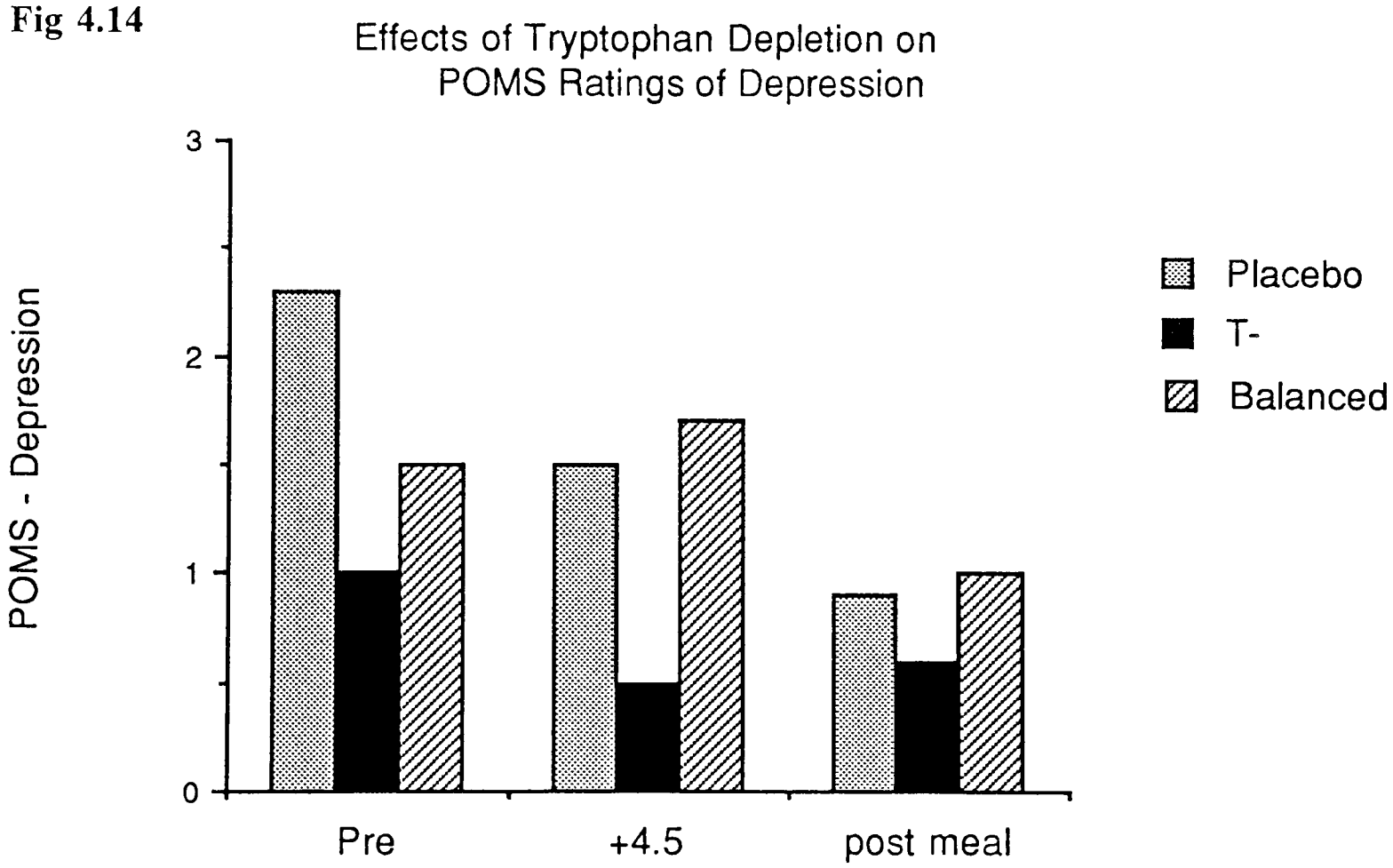


Fig 4.14



An analysis of longer-term changes in mood was also carried out using the data collected from the take-home booklets which assessed the rest of the test session day and the following day. However, as a result of poor compliance with completion of VAS diaries, only six complete sets of VAS and POMS data were available for this analysis; in order to evaluate whether there was a longer term effect on mood, the POMS scores for test day evening and midday of the following day were analyzed. There was a non significant effect of treatment ($F_{2,12}$=1.82, p=0.2), and of treatment by time ($F_{2,12}$=0.73, p=0.50) (Data not shown). In addition to this, none of the VAS mood ratings approached significance, suggesting that the drink had no longer term impact on mood.

In order to assess whether there was any correlation between pre-treatment depression scores and effects of tryptophan depletion on mood, BDI scores obtained at the screening interview were correlated with the change in mood scores obtained on the VAS and POMS. None of these correlations approached significance with the exception of the POMS depression ratings in the placebo condition (Pearson's r=-0.51, p=0.05) suggesting that the mood of subjects with high baseline scores correlated with a higher fall in POMS depression ratings across the initial test morning.

4.4 DISCUSSION

Biochemical Measures. This study confirmed the efficacy of a lower dose of amino acids to lower plasma tryptophan over 4.5 hours. This dose was well tolerated, and not associated with nausea or vomiting. The effects of this dose on tryptophan lowering proved to be highly comparable with the 100g dose of Young et al (1985). The 50g dose yielded a 78% decrease in total plasma tryptophan compared with a decline of 76% in the Young et al (1985) study. Similarly, plasma free tryptophan fell by 67% compared with Young et al's 60% decline. There were some differences in the balanced condition where the lower dose was not associated with changes in total or free tryptophan compared with Young et al who had increases of 72% and 84% respectively. The addition of a placebo control demonstrated that both total and free tryptophan levels fall across a morning, presumably an effect of fasting. Whilst these natural fluctuations were small in comparison with the striking decreases associated with the (T-) drink, the inclusion of this control was important in interpreting the effects of the (T-) condition.

Appetite This study found no effect of tryptophan depletion on measures of appetite. Whilst it is often difficult to demonstrate an association between 5-HT depletion and appetite (see Introduction), this lack of effect was surprising. Clearly, only indirect evidence exists to associate this manipulation with 5-HT depletion (Section 2.1), but the data are interesting to consider in the light of the theory of Wurtman and Wurtman (1979a) (Section 1.5.2.3). Here it is argued that the ratio of tryptophan to LNAAs directly influences selection of macronutrients at the next meal. Clearly the striking alterations in the ratio of tryptophan to LNAAs produced by the tryptophan lowering condition, would be expected to produce a shift in macronutrient selection. A basic tenet of this theory is

that the competitive transport mechanism is central to the effects of the regulation of appetite (and carbohydrate intake specifically) by brain 5-HT. This influential model has recently been regarded as seriously flawed (see Fernstrom and Fernstrom, 1988 for review). Clearly, the data from the present study would support this position.

This study did not support the finding of Young et al (1988) who reported that a 50g amino acid dose produced a small but significant decline in protein selection in a buffet test meal. However, Young et al carried out their study on males, and it is possible that our lack of alteration in appetite was related to sex differences. For example, it has been shown that in response to administration of tryptophan (2g and 3g) males reduced their caloric intake, but females did not (Leiter et al, 1987).

Mood. In contrast to our hypothesis and to the studies of Young et al (1985) and Smith et al (1987), there was no effect of tryptophan depletion on mood. However, as discussed above, the balanced drinks in these studies were not comparable with this study because of the large increases in plasma total and free tryptophan seen with the 100g amino acid dose. It is possible that this difference in the balanced conditions may have had a bearing on the psychological data collected; there may have been a greater contrast between the two conditions in the Young et al study because of these differences in tryptophan levels. Another differing factor may be that, with a smaller dose of amino acids, the tryptophan remaining in plasma has rather less competition from other amino acids for transport at the blood-brain barrier (see Section 1.1). It is possible, therefore, that the reduction in tryptophan availability to the brain produced by the 50g dose may not have diminished brain 5-HT function sufficiently to produce a detectable lowering of mood. Despite this, the 50g dose has been used in preference to the larger dose by research groups; for

example, Abbott et al (1992) have demonstrated that this lower dose blocks morphine analgesia.

As discussed above, there may be sex differences to the effects of tryptophan depletion. However, with regard to mood, it was expected that females would be more vulnerable than males. Previous research has demonstrated, for example, that while a calorie-restricting diet lowers plasma tryptophan concentrations in both males and females, only in females was this reduction in tryptophan availability associated with altered brain 5-HT function (Anderson et al, 1990b, Goodwin et al, 1987). Similarly, Delgado et al (1989) found that a diet that moderately restricted tryptophan intake (700mg/day) altered brain 5-HT function in women but not in men, while a more stringent reduction in dietary tryptophan intake (200mg/day) altered brain 5-HT function in both men and women.

Variations in the menstrual cycle are known to alter mood and appetite (Goodall et al, 1991b; Bowen and Grunberg, 1990). It is possible, therefore, that the effect of tryptophan depletion in females could be confounded by variations in the menstrual cycle. However, the test sessions were not carried out in the pre-menstrual phase of the cycle, and there was no evidence of systematic bias in the allocation of particular drinks to a specific phase of the cycle.

An important factor in the lack of effect with tryptophan depletion on mood may be the lack of vulnerability of the subject group studied. All volunteers were carefully screened and, in general, had very low depression scores prior to entry into the study. Young reports that several of the subjects in studies showing a depressive effect of tryptophan depletion had pre-treatment depression ratings at the higher end of the normal range (pers comm).

This may well have increased their vulnerability to the mood-lowering effect of tryptophan depletion (Abbott et al, 1992). This proposal is strengthened by the findings of Delgado et al (1990) that the tryptophan-free amino acid drink produced an acute relapse in depressive symptomatology in patients recently recovered from a severe depressive episode. It is possible, therefore, that a depressive mood reaction to tryptophan depletion is an indication of a more general vulnerability to develop depressive symptomatology. However, the correlational analyses carried out for the present study did not provide any evidence that those subjects with higher BDI scores at entry were more vulnerable to the effects of tryptophan depletion on mood.

In conclusion, the present study confirms that a 50g amino acid loading reliably lowers plasma total and free tryptophan. However, in carefully screened healthy female subjects, there did not appear to be any effects of this procedure on mood, appetite or food intake. But recent studies suggest that the psychological effects of tryptophan depletion may be present in subjects with higher degrees of vulnerability to affective symptomatology (Miller et al, 1992). This is of particular importance to the present thesis; there is a well established association between the development of eating disorders and affective symptomatology, an association which is most robust in bulimia nervosa (see Section 1.3.2.2 for review). Furthermore, the association between eating disorders and dysregulation of brain 5-HT (see Section 1.3.3) suggests that an eating-disordered population may be of particular risk to a state of tryptophan depletion. It would therefore be important to study a group of subjects who were at risk of developing, or had previously developed an eating disorder in order to establish whether this vulnerability existed. This issue will be addressed more fully in the subsequent study.

CHAPTER 5 A COMPARISON OF THE EFFECTS OF TRYPTOPHAN DEPLETION BETWEEN HEALTHY FEMALE VOLUNTEERS AND WOMEN WHO HAVE RECOVERED FROM BULIMIA NERVOSA

5.1 INTRODUCTION

Bulimia nervosa has been strongly associated with impaired brain 5-HT function (see section 1.3.3 for review). For example, metabolite studies demonstrate that bulimic patients have lower CSF 5-HIAA levels than controls (Jimerson et al, 1988; Kaye et al, 1990). In addition, the uptake of 5-HT into blood platelets appears to be abnormally increased in symptomatic patients (Goldbloom et al, 1990), suggesting an analogous decrease in 5-HT neurotransmission in the brain. Neuroendocrine data provide further evidence of impaired brain 5-HT function in bulimia; blunted PRL responses have been reported in response to the 5-HT receptor agonist, mCPP (Brewerton et al, 1992), suggesting hyposensitivity of brain 5-HT receptors.

It has further been argued that behavioural symptoms associated with bulimia are consistent with hyposerotonergic activity (Kaye et al, 1991). Firstly, hyposerotonergic function is consistent with mood impairment and depression; affective disorder is commonly associated with bulimia, and bulimic patients have a high lifetime risk for development of major depression (see section 1.3.2.2 for review). Secondly, it has been shown that interventions that diminish 5-HT neurotransmission or receptor activation are associated with an increase in food consumption (see section 1.5.2.3 for review), thus theoretically linking bingeing behaviour with hyposerotonergic function and associated alterations in the satiety response.

This is further supported by data which demonstrate that the selective 5-HT re-uptake inhibitor, fluoxetine, reduces the frequency of binge eating in bulimic patients (Fluoxetine Bulimia Nervosa Collaborative Study Group, 1992).

Furthermore, it has been observed that binge eating is frequently associated with the consumption of carbohydrate-rich foods. It has been argued that the consumption of large amounts of carbohydrate foods is a way in which uptake of tryptophan to the brain may be enhanced. Carbohydrate consumption is known to cause insulin secretion which in turn depresses plasma levels of most LNAAs, but not tryptophan (Fernstrom and Wurtman 1972). Consequently, the ratio of tryptophan to other LNAAs is increased, thus enhancing transportation across the blood-brain barrier and synthesis of brain 5-HT (section 1.1.2). Thus, it has been argued that binge eating may be a way of restoring 5-HT function in individuals with bulimia (Kaye et al, 1988). It would therefore be important to assess the macronutrient content of food consumed in response to tryptophan depletion.

Cases of clinical eating disorders, including bulimia, usually begin as normal dieting which then gets out of control (Hsu, 1990b). The study reported in Chapter 3 of the present thesis demonstrated that moderate dieting is associated with a lowering of plasma concentrations of tryptophan, and previous studies have demonstrated that this is associated with alterations in brain 5-HT function in women but not in men (see section 1.4.3). It has therefore been hypothesised that these dieting-induced reductions in plasma tryptophan, and associated decrease in brain 5-HT function may be a key neurobiological mechanism which contributes to the development of eating disorders in vulnerable subjects (Cowen et al, 1992).

The previous two studies of the present thesis were carried out on carefully screened individuals who were not at risk of either affective or eating disorders. Thus the tryptophan reductions seen in these studies would be unlikely to be associated with marked behavioural changes. The present study, therefore, attempts to carry out acute tryptophan depletion in a group which is likely to be vulnerable to the behavioural effects of a sudden reduction in brain 5-HT function. It has previously been demonstrated that this manoeuvre is associated with a rapid lowering of mood in patients recently recovered from depression (Delgado et al, 1990). The present study, therefore, carried out acute tryptophan depletion in a group of patients who had previously suffered from bulimia but who had now recovered.

The hypotheses for this study were that tryptophan depletion would be associated with a return of depressed mood, and that recovered bulimic (RBN) subjects would demonstrate increased food consumption at a test meal with increased ratings of disturbed eating associated with binge eating (eg fear of loss of control, preoccupation with food). It was further hypothesised that an increase in appetite would be characterised by an increase in carbohydrate consumption relative to other macronutrients. The study was designed so that the responses of the recovered bulimic subjects could also be compared with those of the healthy female volunteers reported in the previous chapter.

5.2 METHODS

Volunteers

Volunteer recruitment and characteristics for the 12 healthy female volunteers are set out in section 4.2. Nine women who had previously had an episode of bulimia nervosa were recruited for this study. This was done by advertisement and by approaching cases identified by case notes via GP practices. In the latter instance, cases were identified through the Department of Clinical Psychology. GPs were then contacted, together with the treating psychiatric professional where applicable. Volunteers were only contacted if the GP and other psychiatric professionals involved agreed to this. Volunteers were asked if they wished to participate in a study looking at the effects of a tryptophan-free amino acid drink on mood and appetite. Potential volunteers were invited to the research unit where full details of the study were given, and an opportunity to ask questions was provided. Volunteers were screened in the same way as the control group (section 4.2), although they were not excluded for history or family history of affective disorder. In addition to the standard screening and physical examination, a clinical interview was carried out to gain a full history of the eating disorder, and of current status. Volunteers were required to have met DSM-III-R criteria for bulimia nervosa, but to be recovered. Recovery was defined as a period of at least six months during which there were no episodes of vomiting or laxative abuse, not more than one objective episode of binge eating per month, and not more than one episode of subjective bingeing per week. Binge episodes were assessed using the EDE-Q3 in addition to the clinical interview which was carried out by a psychiatrist specialising in the area of eating disorders. Volunteers were excluded if there was any indication of other eating disorders, and if they had a BMI of below 20. The

EDE-Q3 questionnaire subscales which assessed restraint and attitudes to body weight and shape were also included (Cooper and Fairburn, 1987).

EAT (Garner and Garfinkel, 1979) and BDI (Beck et al, 1961) scores were collected, although they were not used as exclusion measures unless they indicated current psychiatric disorder in conjunction with the clinical interview. All volunteers were physically well and had no ongoing psychiatric illness. All volunteers had a regular 28 day menstrual cycle, other than one volunteer who was post-menopausal, and one who had undergone a hysterectomy.

One volunteer from the RBN group was not included in the data analysis of this study, as it was clear from the analysis of plasma tryptophan that she had been given two tryptophan-deficient drinks to the exclusion of the balanced drink.

Experimental Design

The design of the experiment was a between- and within-groups comparison. Between-groups comparisons were carried out on all measures taken within sessions to enable direct comparison of the RBN group with a healthy control. In addition, for repeat measures, within-subjects comparisons were made in order to assess the effects of condition and time. The RBN group recorded additional mood and appetite ratings over the week following each experimental session, and these data were analyzed separately in a within-subjects fashion. The study was controlled for menstrual cycle (see procedure).

Dietary Manipulation

Plasma tryptophan was lowered using the 50g amino acid loading technique outlined in the general methods chapter (section 2.1). This procedure was identical for the two groups.

Procedure

The design remained identical for the RBN group such that subjects were required to attend the research unit for three complete mornings, each a minimum of one week apart. In the RBN group, these sessions were carried out between days 3-21 of the menstrual cycle to avoid the luteal phase of the cycle. To do this, the three experimental sessions were carried out over two consecutive menstrual cycles. It was ensured that no systematic bias to a particular phase of the cycle was employed in the allocation of these sessions.

Recovered bulimic subjects were required to attend the unit for three experimental sessions in order to complete the study successfully. The timetable for each experimental session remained identical to that employed for the healthy female study; the procedure for this is set out in section 4.2. In addition to this, volunteers in this group were asked to keep diaries for the rest of the test session day and the following five days. These included VAS ratings of mood and appetite, POMS rating scales and a BDI (McNair et al, 1971; Beck et al, 1961). This diary replaced the two day follow-up procedure used in the healthy control study. The diary was based on this, but was extended to follow participants for a total of 6 days. Thus, POMS rating scales were inserted for the first two days along with the VAS rating scales. The BDI was inserted at the end of the diary, and asked participants how they had been feeling over the past week.

Validity of comparison

12 healthy female volunteers were compared with eight women who had recovered from a clinical episode of bulimia nervosa. The within-session data for these two studies had been collected in the same way, thus enabling direct comparison of the data. The data collected after the test session was carried out in a similar fashion with take-home diaries. However, as the methodologies differed in terms of the data collected out of the experimental session (ie RBN subjects kept VAS diaries for six days compared with two, and this included extra ratings such as a BDI), comparisons were not made for this period. Despite these slight differences in methodology, it seemed very unlikely that this would introduce a bias in the data collection.

Measures

Mood and appetite were assessed in an identical fashion to the healthy control group. The measures adopted are set out in section 4.2 in the previous chapter. (See Appendices 2.6-11, 3.3 and 4.1).

Restraint and 'attitudes to body weight and shape' were measured using the relevant subscales of the questionnaire form of the EDE-Q3 (Cooper and Fairburn, 1987) (Appendix 5.1).

Diaries for this study contained the same measures adopted for the previous study for follow-up for the test day, and the next day. This was, however, modified to assess subjects over a total of six days compared with two days in the previous study. Daily VAS

ratings were included for measurement of mood, appetite, and eating disturbance (Appendices 2.6, 2.10 and 5.2). The instructions for these were modified, where appropriate, to assess the individual over the whole day. A POMS questionnaire (Appendix 2.11) was included for the end of the test session day and the following day. This had been amended to assess the last 24 hours. A one-week BDI was included at the end of the week to assess levels of depression over the post-session week.

Biochemical Methods

Levels of plasma tryptophan were determined from 10ml baseline fasting blood samples, and samples taken at +4.5 hours. All samples were taken into heparin tubes. This was carried out by research nursing staff. Plasma samples were separated by centrifugation and stored at -30°C. Plasma total and free tryptophan were determined by fluorimetric detection according to the method of Bloxam and Warren (1974), and Bloxam et al (1977). Methodology for assay procedures of tryptophan are set out in section 2.4.

Data Analysis

The data were analyzed by repeated measures analysis of variance (ANOVA) using the BMDP P2V statistical package. This analysis was group (normal vs RBN) by condition (placebo vs T- vs Balanced). Greenhouse Geisser probability was used where measures were repeat.

5.3 RESULTS

Subjects

The group characteristics for the eight recovered bulimic volunteers are set out in table 5.1 alongside the 12 healthy female controls.

TABLE 5.1: GROUP ASSIGNMENT: BULIMIC VS CONTROL: MEANS AND STANDARD DEVIATIONS

MEASURE	BULIMIC (MEAN ±SD)	CONTROL (MEAN ±SD)
BMI	24.2 (±3.3) Range 21.1 - 29.1	23.0 (±3.4) Range 17.4 - 29.0
BDI	8.2 (±8.21) Range 2 - 25	6.4 (±6.2) Range 0 - 17
AGE	32.9 (±9.45) Range 24 - 52	22.5 (±2.8) Range 19 - 28
EAT	13.8 (±13.7) Range 6 - 43	9.6 (±7.0) Range 2 - 20
RESTRAINT	6.1 (±6.3) Range 0 - 18	7.1 (±4.8) Range 0 - 13
ATTITUDES TO BODY WEIGHT AND SHAPE	32.9 (±15.0) Range 20 - 52	26.5 (±16.5) Range 1 - 61

Unpaired t-tests were carried out on these data. The mean age of the RBN subjects was significantly higher than the control group ($t=3.09$, $p<0.05$, $df=18$), but there were no significant differences on the remaining measures.

Clinical interview and assessment confirmed that none of the RBN subjects had received treatment with psychotropic medication within the previous year. Six subjects had a previous history of DSM-IIIR affective illness, and three had a family history of affective illness. Six subjects had normal menstrual cycles, one had undergone a hysterectomy, and one other was post-menopausal.

Biochemical Measures

Plasma total and free tryptophan were compared for both groups across the three conditions (placebo, T-, and balanced drinks).

For plasma total tryptophan the ANOVA revealed a significant main effect of group ($F_{1,16}=11.32$, $p<0.005$). Post hoc t-tests showed that the RBN group had significantly lower plasma total tryptophan at baseline on each of the three testing occasions. In addition, their total plasma tryptophan was lower than controls 4.5 hours following both the T- and Balanced drinks. There was a significant main effect of condition ($F_{2,32}=60.28$, $p<0.00001$), and a significant condition by time interaction ($F_{2,32}=64.33$, $p<0.00001$), showing a marked effect of the T- drink to lower plasma tryptophan at 4.5 hours (Table 5.2, Fig 5.1a/b). There was, however, no significant interaction of group with time or of group by time by condition (all p values >0.1).

For plasma free tryptophan the ANOVA also revealed a significant effect of condition

Fig 5.1a

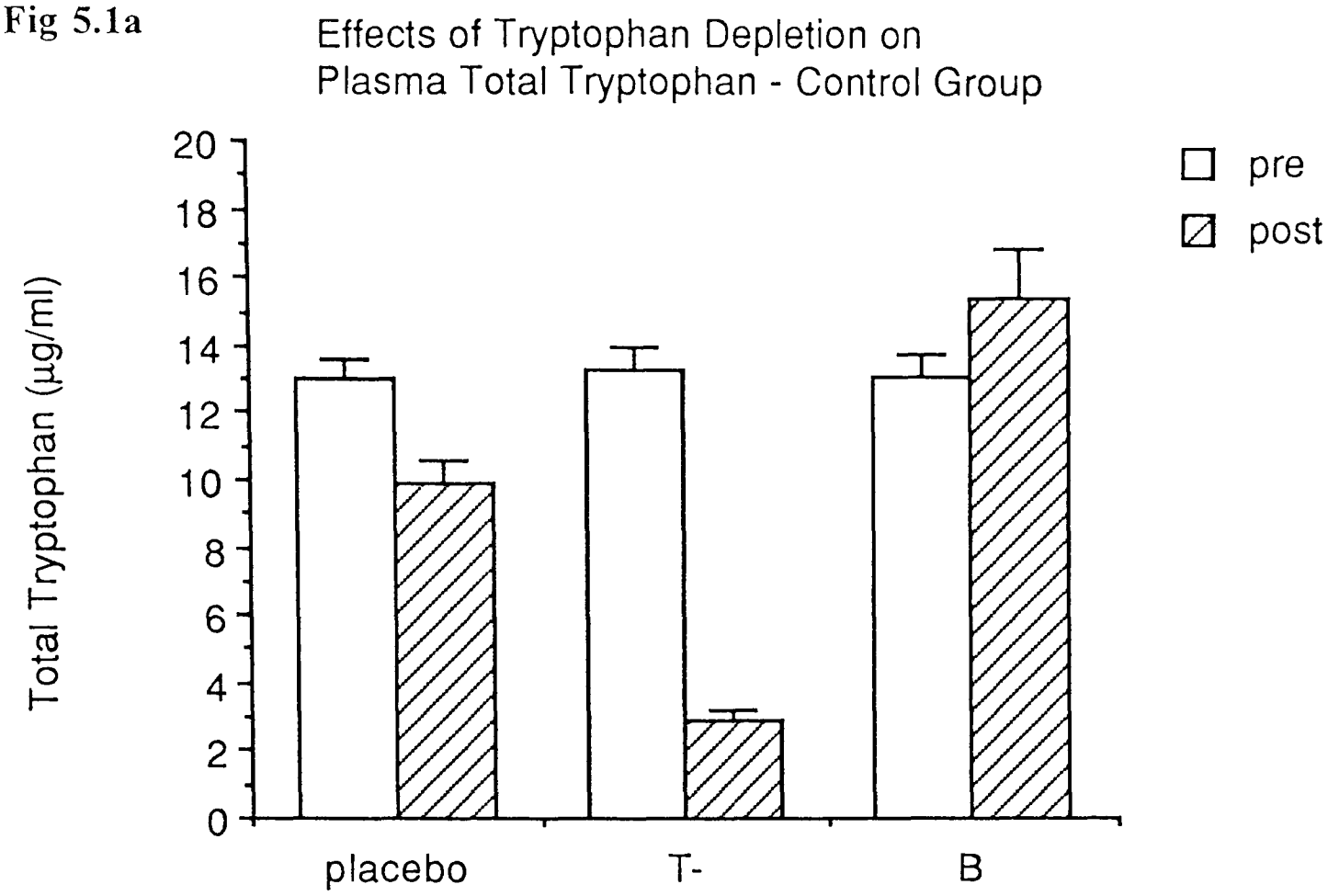
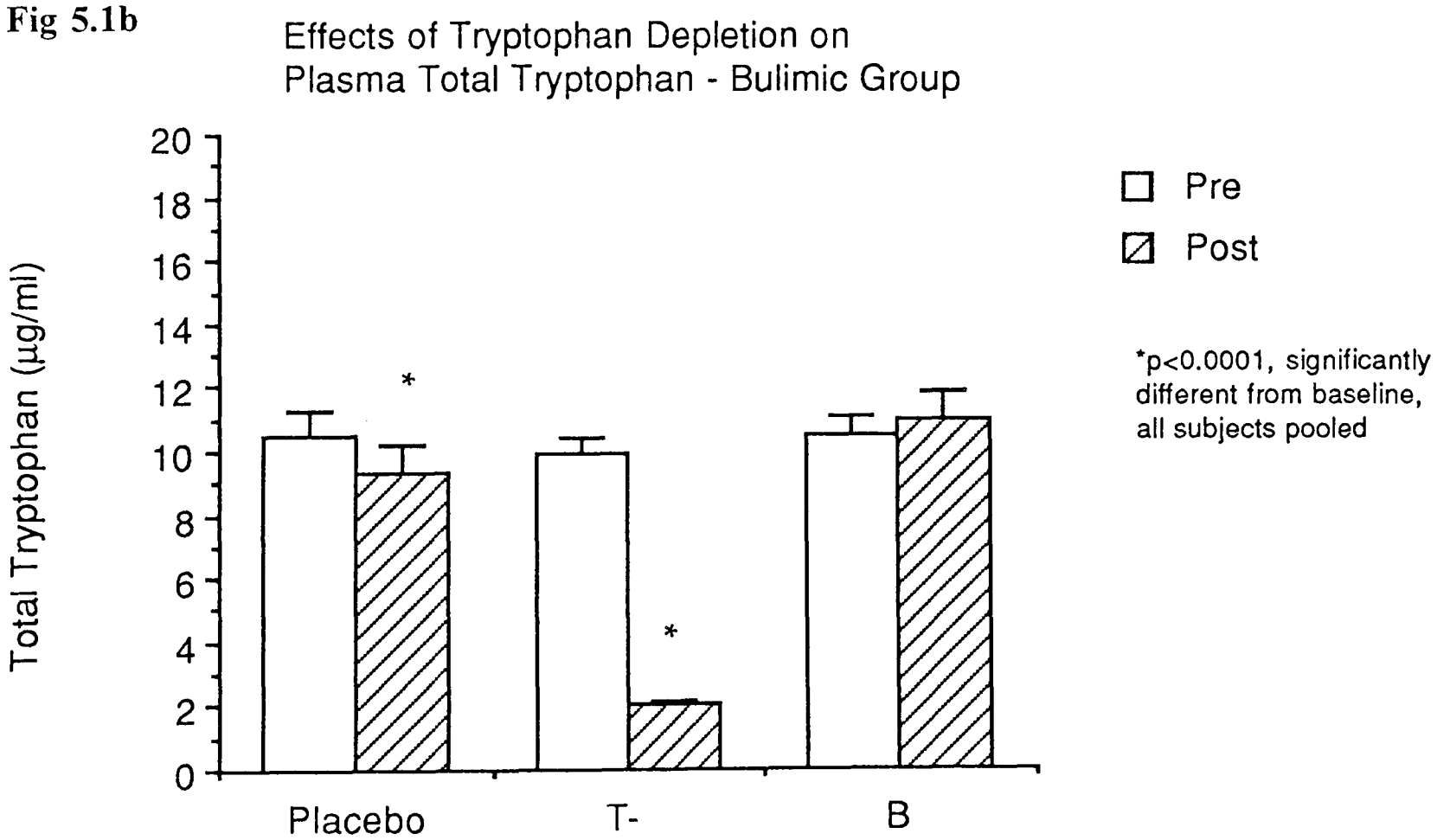


Fig 5.1b



($F_{2,32}$=19.97, p<0.00001), and a significant condition by time interaction ($F_{2,32}$=69.15, p<0.00001), with plasma free tryptophan also being markedly lowered 4.5 hours after ingestion of the T- drink (Table 5.2, Fig 5.2a/b). Here there were also no significant group by time, or group by time by condition interactions (all p values >0.1).

Measures of appetite

The effects of tryptophan depletion on appetite were analyzed by ANOVA in order to examine the effects of tryptophan depletion on subjective ratings of hunger and satiety. This was carried out on appetite related VASs, food preference questionnaires, and test meal data for both groups. The ANOVA findings for these measures are set out in Table 5.3.

VAS Ratings. For VAS ratings of hunger taken across the test session, there was a significant effect of time with hunger rising across the morning ($F_{6,96}$=43.59, p<0.00001). There was a significant main effect of condition (Tables 5.5 and 5.6), but no condition by time interaction ($F_{12,192}$=2.92, p>0.05). The effect of condition appears to be a result of a significant suppression of hunger in both the amino acid conditions (Fig 5.3). There was no main effect of group, and no group by condition interaction.

VAS ratings of satiety were taken before and after the test meal (ie at +4.5 hours after the drink, and immediately after the test meal). The effect of the test meal on these measures is therefore indicated by the time condition on the ANOVA. The parameters here were: wish to eat, feel full, amount could eat, satisfaction of meal.

Fig 5.2a

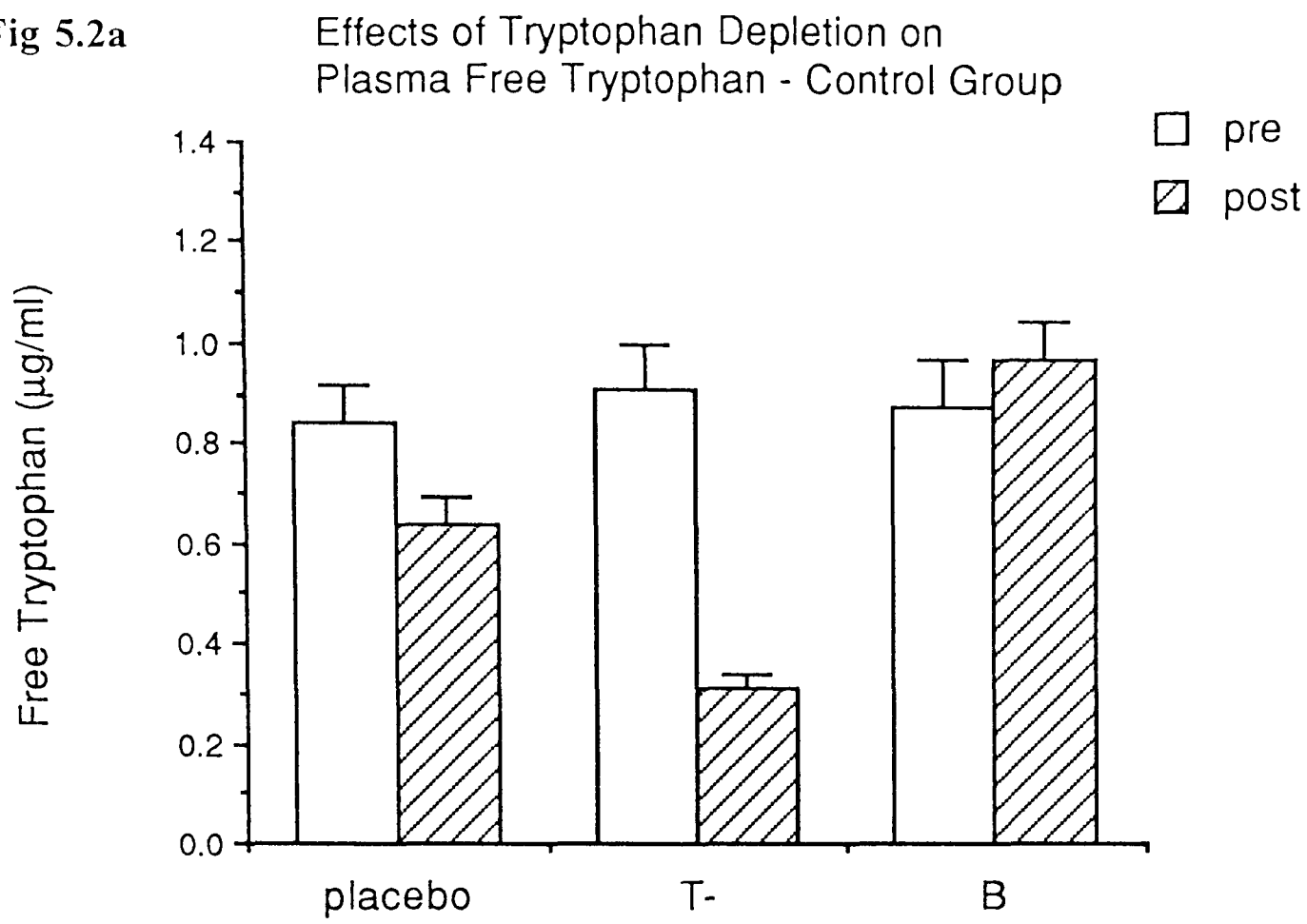


Fig 5.2b

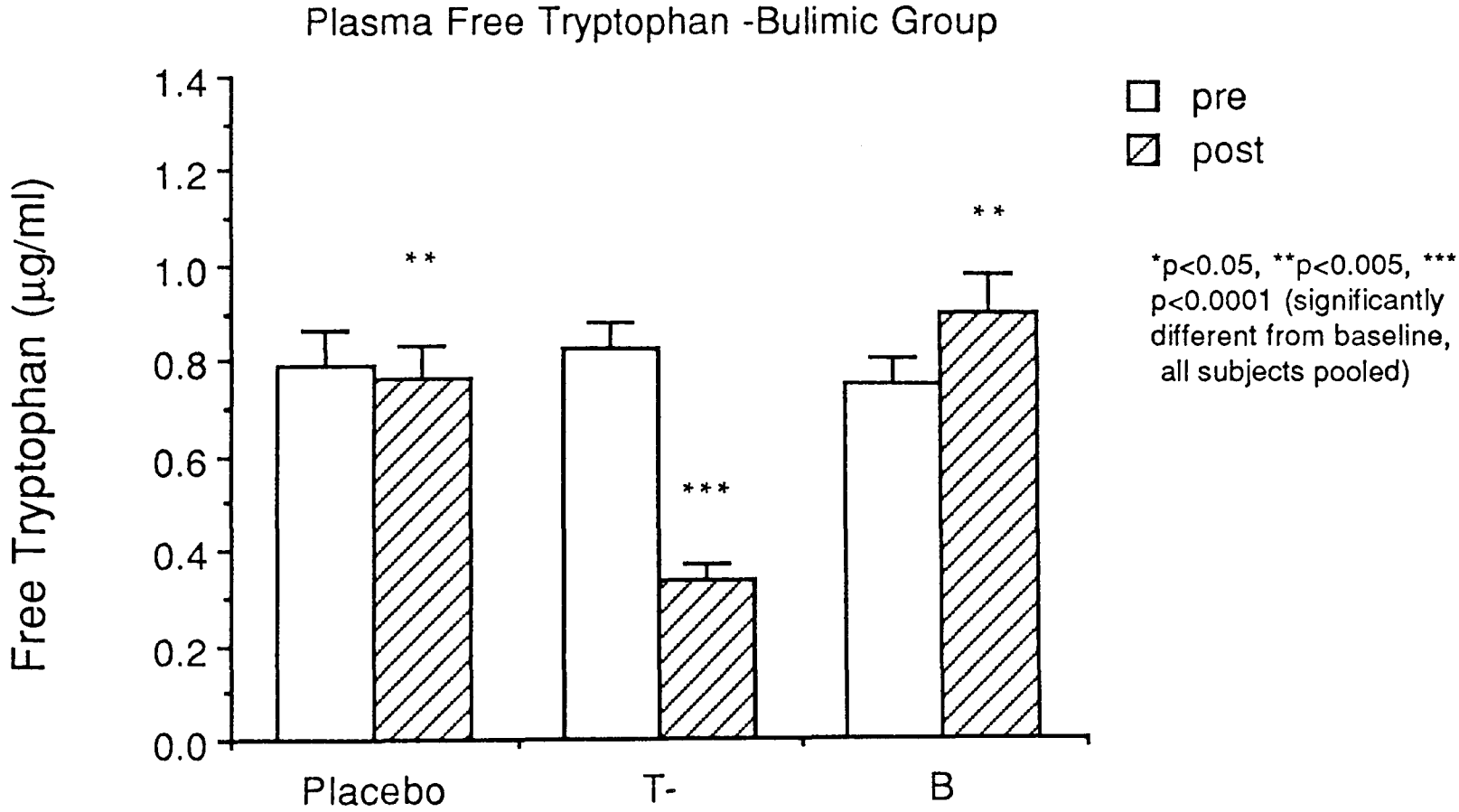
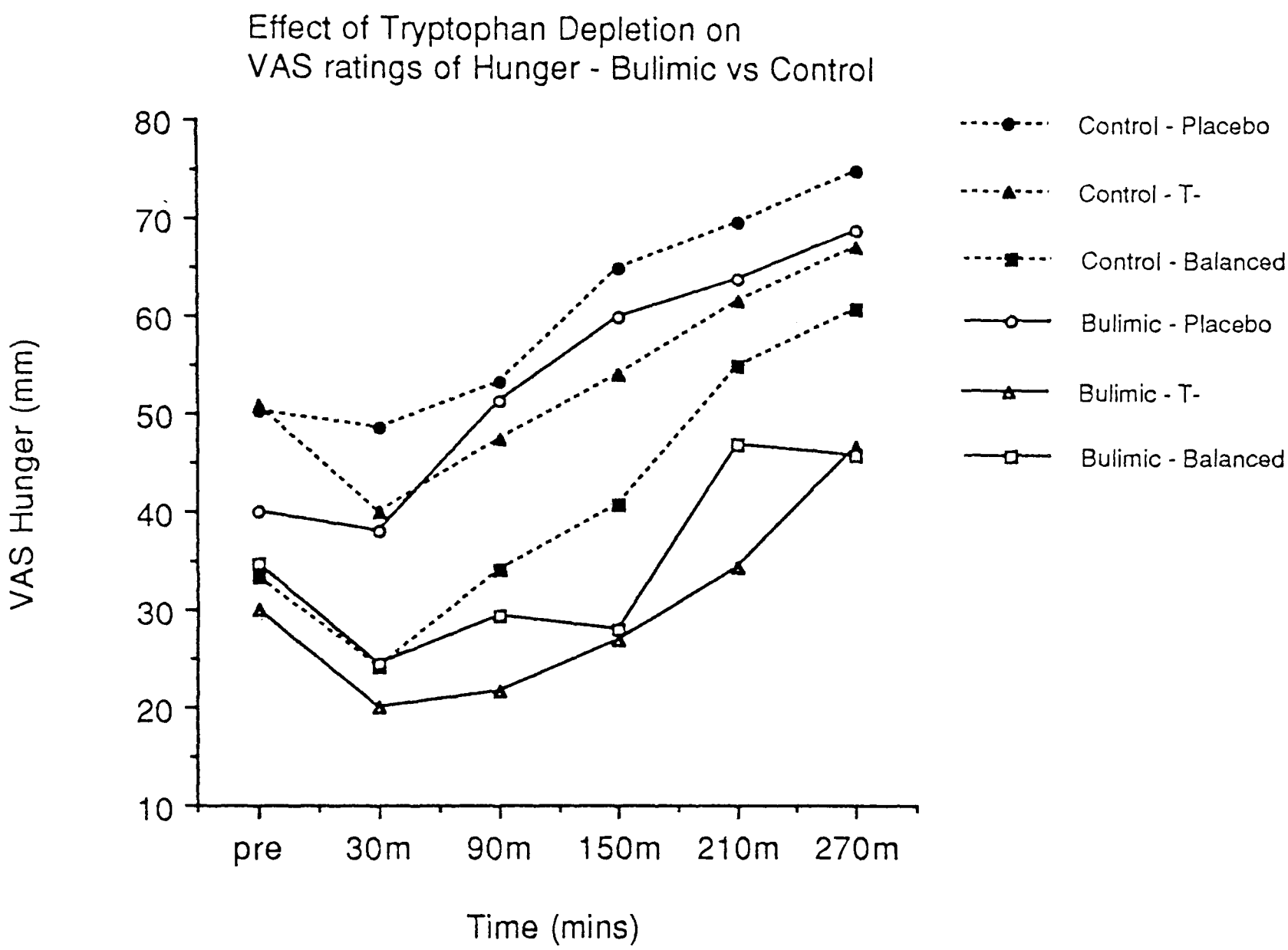


Fig 5.3


Effect of Tryptophan Depletion on
VAS ratings of Hunger - Bulimic vs Control
VAS Hunger (mm)
80
70
60
50
40
30
20
10
pre
30m
90m
150m
210m
270m
Time (mins)
Control - Placebo
Control - T-
Control - Balanced
Bulimic - Placebo
Bulimic - T-
Bulimic - Balanced

As expected, ratings of 'wish to eat' were lowered following the test meal (Tables 5.3 and 5.4). There was, in addition, a significant main effect of condition whereby the two amino acid drinks suppressed wish to eat prior to the meal. This analysis reached significance, although the control subjects, when analyzed alone, produced only a trend ($p=0.07$, see Chapter 4, page 163). This is a result of the increased statistical power generated by a larger group. There was also a significant group by time interaction ($F_{1,16}=5.26$, $p<0.05$) with the RBN group expressing less wish to eat than the controls prior to the test meal, but a greater wish afterwards (Fig 5.4).

Feelings of fullness were significantly increased by the meal ($F_{1,16}=182.3$, $p<0.0001$), but none of the remaining effects of time, or time interactions were significant (all p values >0.1). For VAS ratings of satisfaction of meal, there was a significant main effect of group. This was a result of higher ratings of satisfaction of meal in the control group (Table 5.4). Ratings of 'could eat a large amount of food' were significantly lowered by the meal ($F_{1,16}=74.9$, $p<0.0001$), and there was a significant group by time interaction ($F_{1,16}=11.35$, $p<0.005$), and group by time by condition interaction ($F_{2,32}=5.23$, $p<0.05$). Post-hoc t-tests revealed that the RBN group scored significantly less than the controls on this rating pre-meal following both T- and placebo treatment (Fig 5.5).

Taken together, these data do not indicate any specific effect of tryptophan depletion on ratings of appetite in either the RBN group or controls. However, both amino-acid drinks appeared to lower the wish to eat.

Fig 5.4

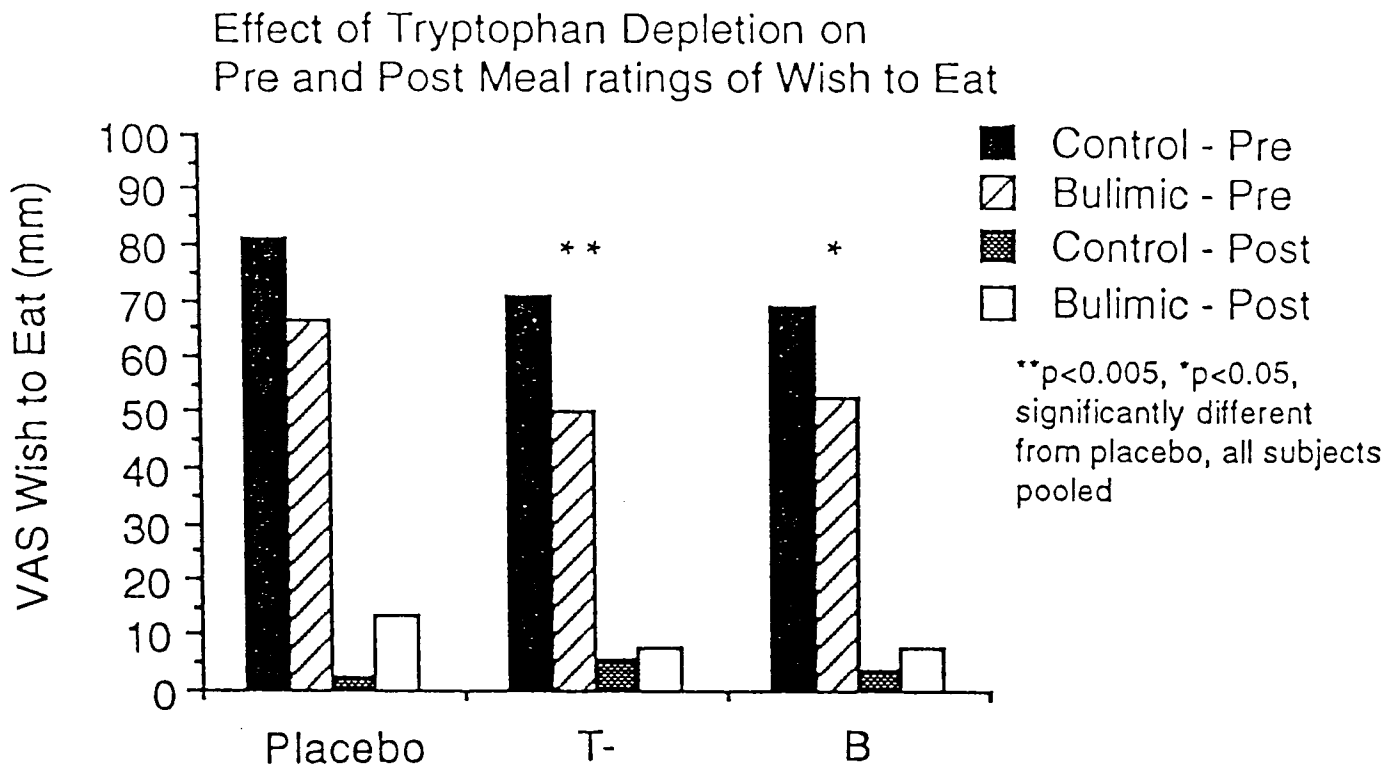
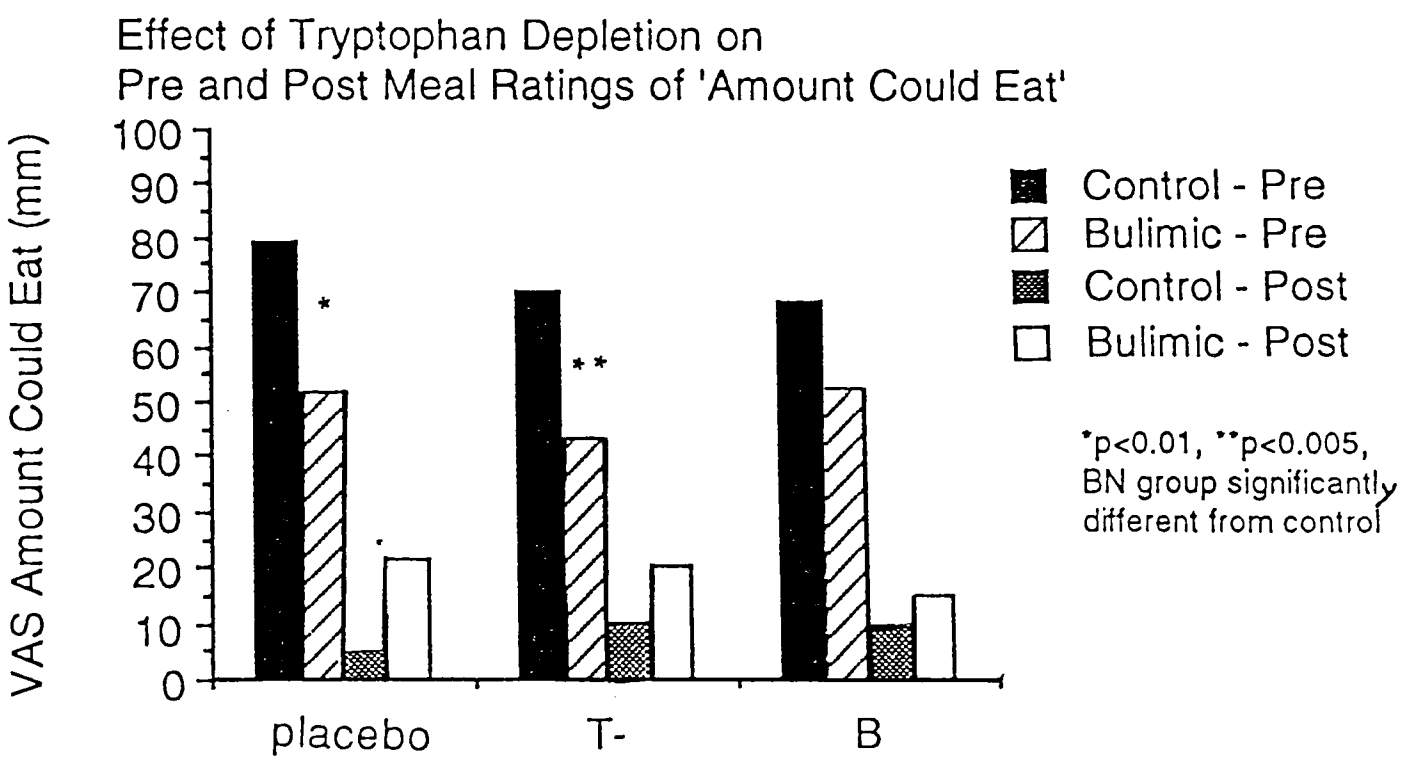

Effect of Tryptophan Depletion on
Pre and Post Meal ratings of Wish to Eat
VAS Wish to Eat (mm)
100
90
80
70
60
50
40
30
20
10
0
* *
*
Placebo
T-
B
Control - Pre
Bulimic - Pre
Control - Post
Bulimic - Post
**p<0.005, *p<0.05,
significantly different
from placebo, all subjects
pooled

Fig 5.5


Effect of Tryptophan Depletion on
Pre and Post Meal Ratings of 'Amount Could Eat'
VAS Amount Could Eat (mm)
100
90
80
70
60
50
40
30
20
10
0
*
**
placebo
T-
B
Control - Pre
Bulimic - Pre
Control - Post
Bulimic - Post
*p<0.01, **p<0.005,
BN group significantly
different from control

Food preference questionnaires had been give pre- and post-test meal in addition to a baseline morning rating. These were analyzed to examine the effects of tryptophan depletion on subjective preference of macronutrients in addition to the test meal findings.

For selection of fat items on the free choice measure, there was a significant group by condition interaction (Tables 5.3 and 5.5), and a group by time interaction ($F_{2,32}$=3.43, p=0.05). The group by condition interaction was a result of the increased selection of fat items pre-meal in the placebo and T- conditions in the control group. The group by time interaction was a result of the wide spread at baseline and pre meal, with the recovered bulimics choosing relatively few fat items (Fig 5.6a/b).

For analysis of forced choice of carbohydrate over protein, there was a significant group difference (Tables 5.3 and 5.5). This effect was a result of the relative reduction in choice of carbohydrate items in the RBN group compared with the normal controls.

Test Meal. For total calories consumed at test meal, there was a significant effect of group, and of condition (Table 5.6). Post hoc t-tests revealed that the RBN group consistently ate fewer calories than the normal controls (t=2.41, p<0.05, df=18). The condition effect appeared to be a result of both groups consuming less calories in the placebo condition. There was, however, no group by condition interaction (p>0.05), showing no effect of tryptophan depletion on the calorie intake of the RBN group (Fig 5.7).

Analysis of the macronutrient content of the food consumed revealed a significant main effect of group for total fat intake, where the RBN group consumed significantly less fat than the controls (t=2.39, p<0.05, df=18). There was also a significant main effect of

Fig 5.6a

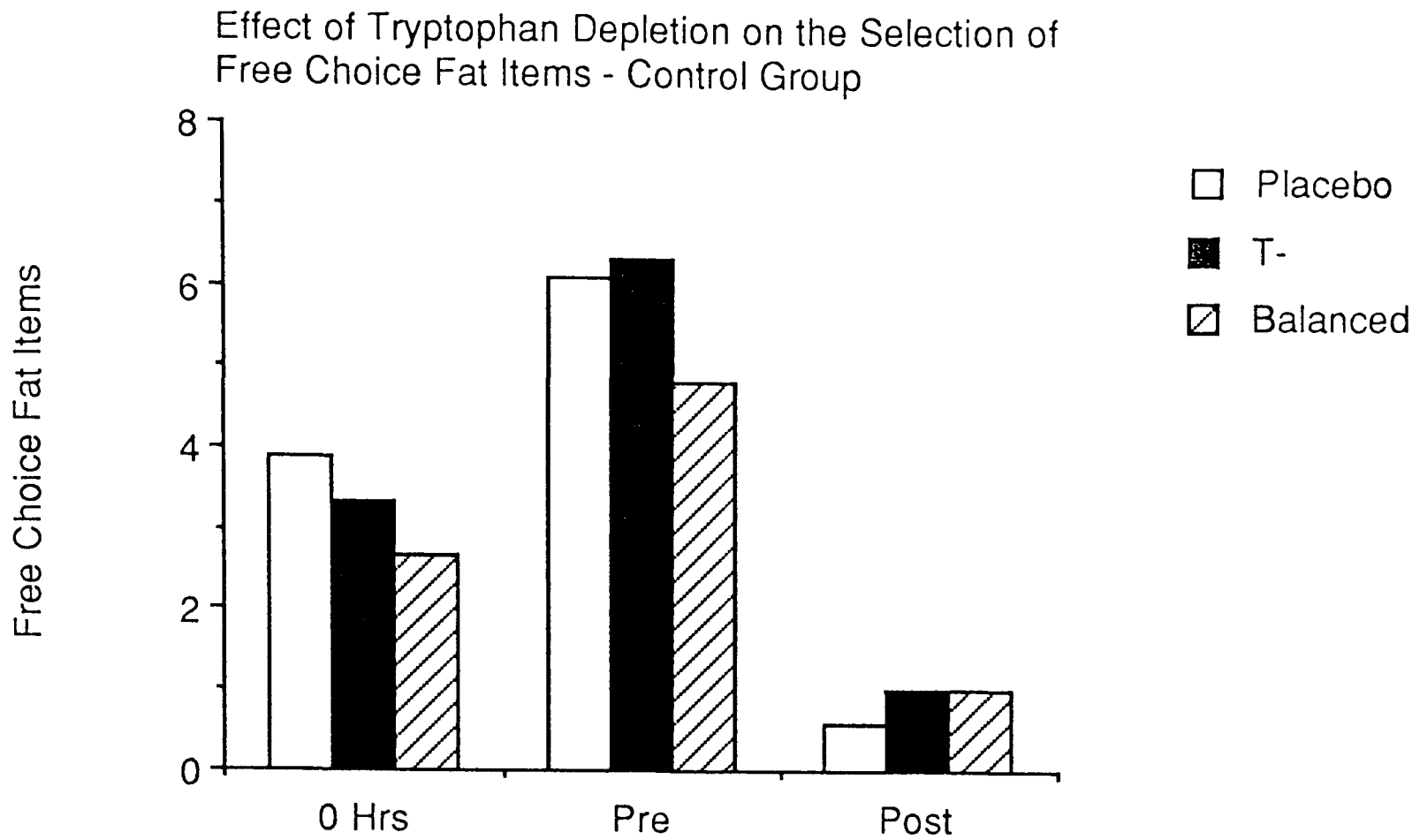


Fig 5.6b

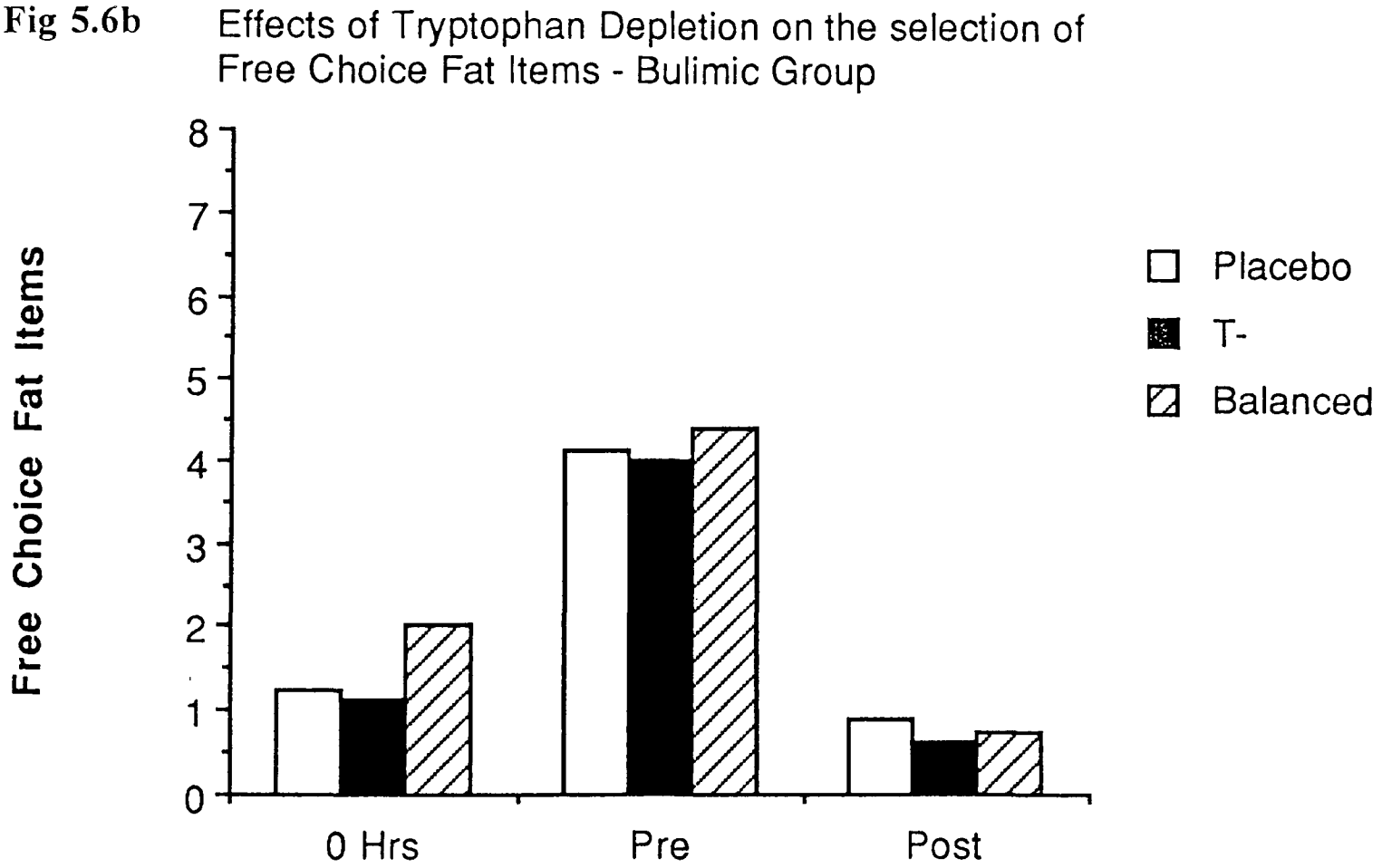


Fig 5.7

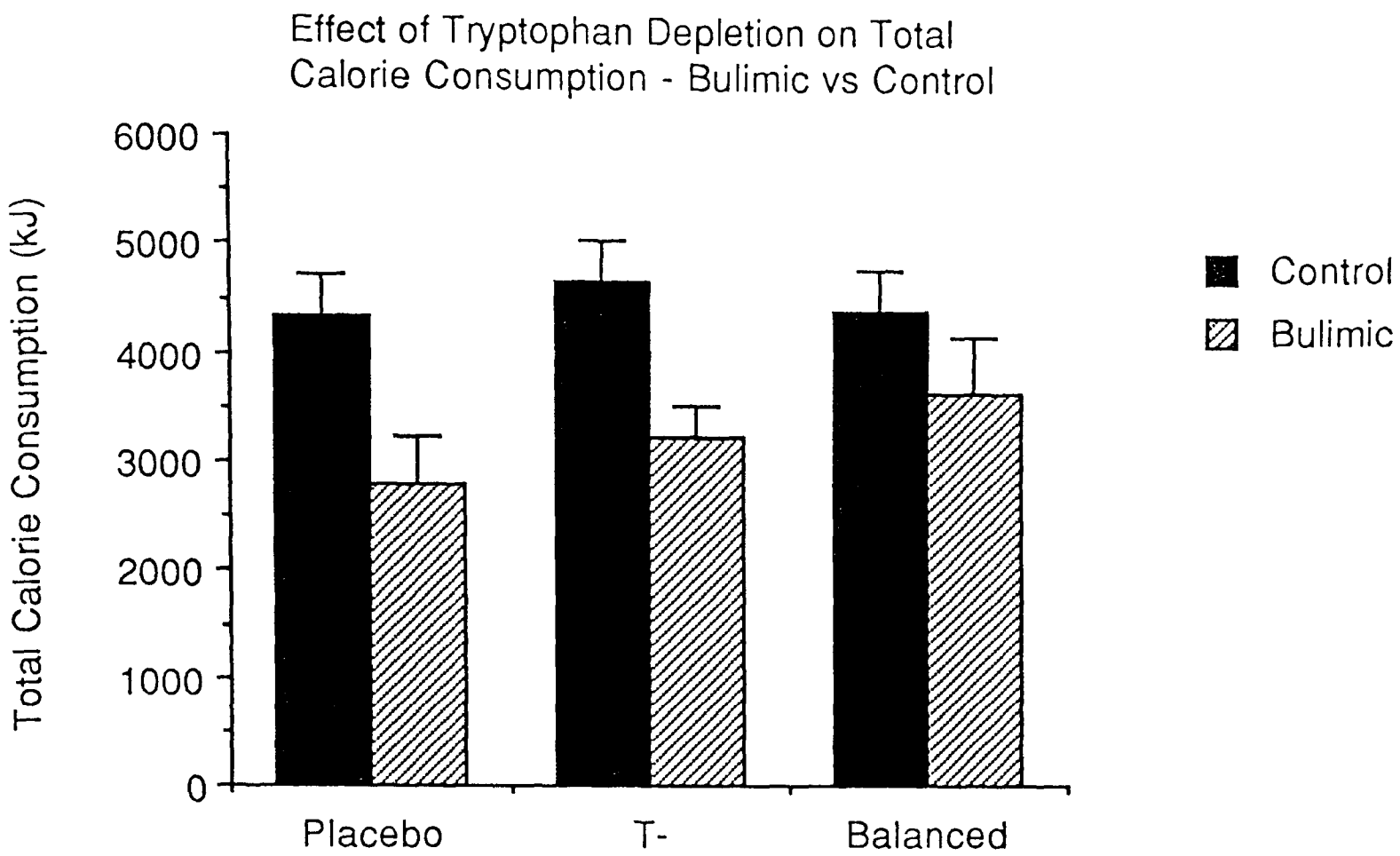
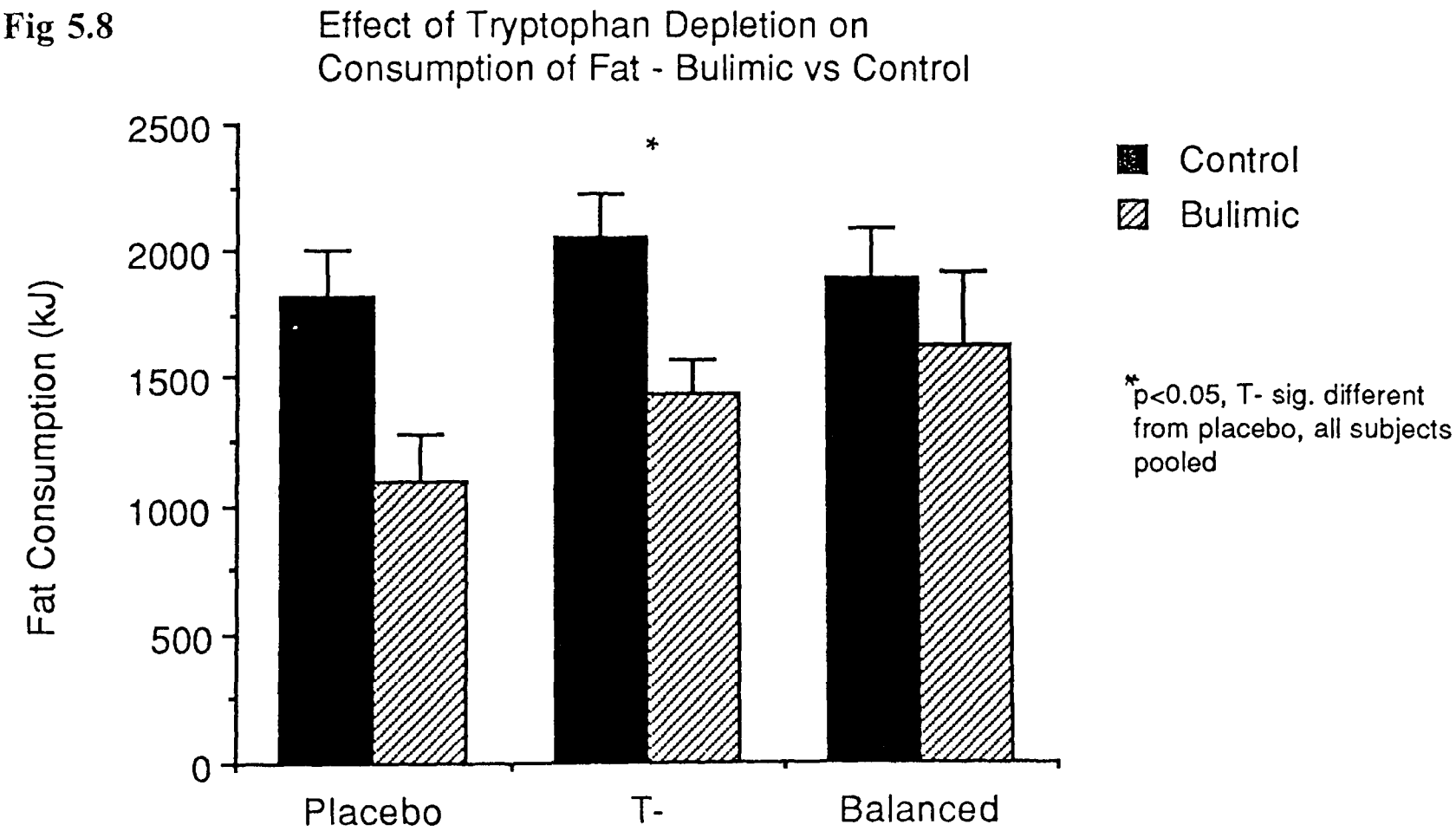

Effect of Tryptophan Depletion on Total Calorie Consumption - Bulimic vs Control
Total Calorie Consumption (kJ)
6000
5000
4000
3000
2000
1000
0
Placebo
T-
Balanced
Control
Bulimic

Fig 5.8


Effect of Tryptophan Depletion on Consumption of Fat - Bulimic vs Control
Fat Consumption (kJ)
2500
2000
1500
1000
500
0
*
Placebo
T-
Balanced
Control
Bulimic
*p<0.05, T- sig. different from placebo, all subjects pooled

condition which, as with total calorie consumption, resulted from a suppression of intake in the placebo condition. There was also a trend for a group by time interaction for consumption of fat (Table 5.6) (Fig 5.8). For protein and carbohydrate consumption, there were no significant main effects or interactions (Figs 5.9 and 5.10).

There were no other significant differences between the RBN group and the control, and the T- drink did not alter either total calorie intake or the intake of specific macronutrients.

Disordered Eating

Measures of disordered eating were analyzed to assess the impact of tryptophan depletion on specific symptoms related to the bulimia syndrome. The ANOVA findings for these measures are set out in Table 5.7.

For VAS measures designed to assess disordered eating, there were significant group differences for 'fear of loss of control', and 'resist the urge to overeat' (Table 5.8), but there were no group by condition or group by condition by time interactions. These data indicate that the greater fear the recovered bulimic subjects had of losing control of their eating was present under all conditions, and was not a consequence of tryptophan depletion (Fig 5.11). Ratings of 'resist the urge to overeat' similarly showed only a significant group difference (Table 5.8). Ratings of 'unable to stop eating' showed neither a significant group difference nor a group by condition interaction ($p>0.1$). These data indicate that the recovered bulimic subjects had increased ratings on several measures of abnormal eating, but that the measures were not influenced by the T- drink.

Fig 5.9

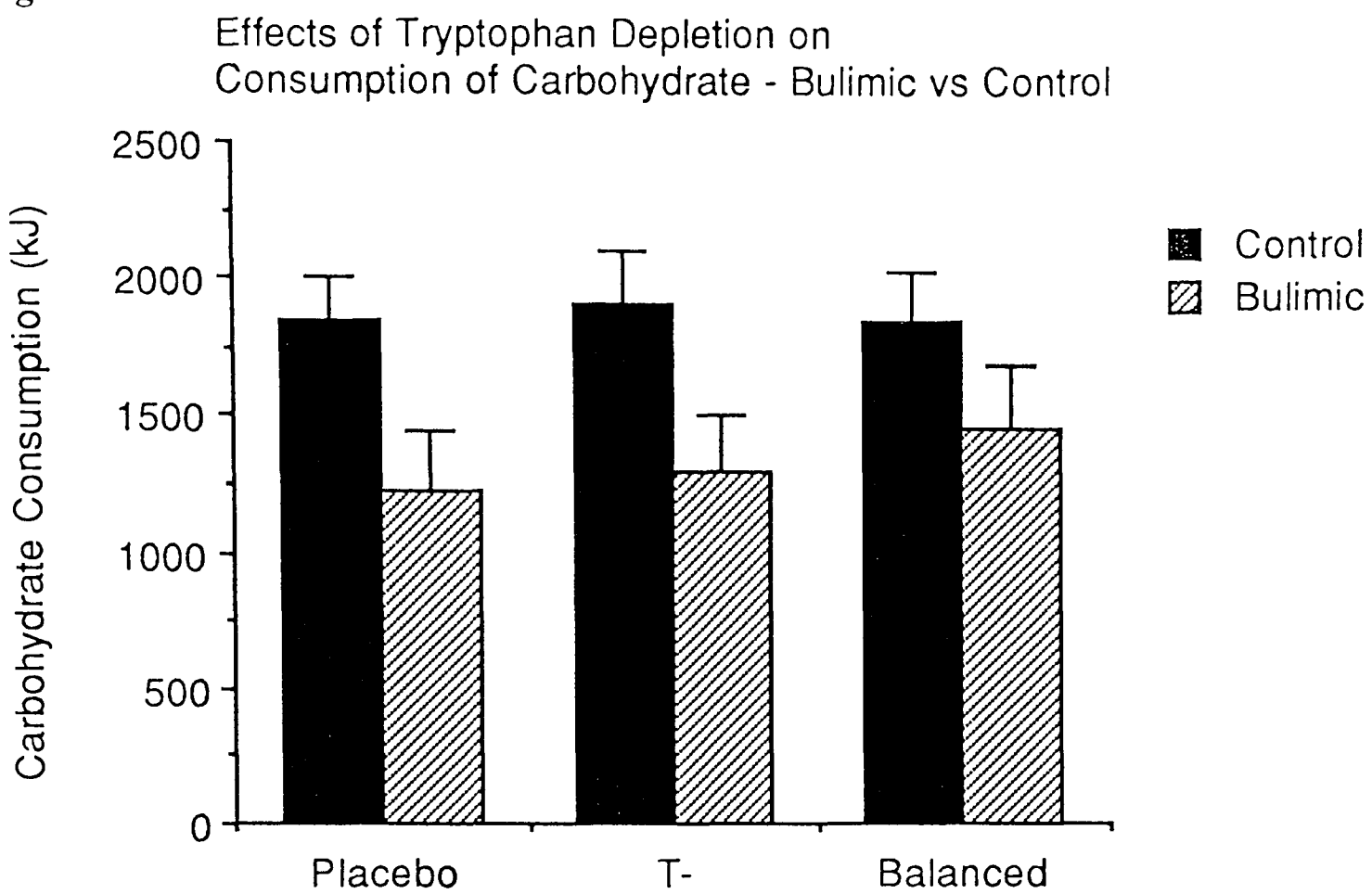


Fig 5.10

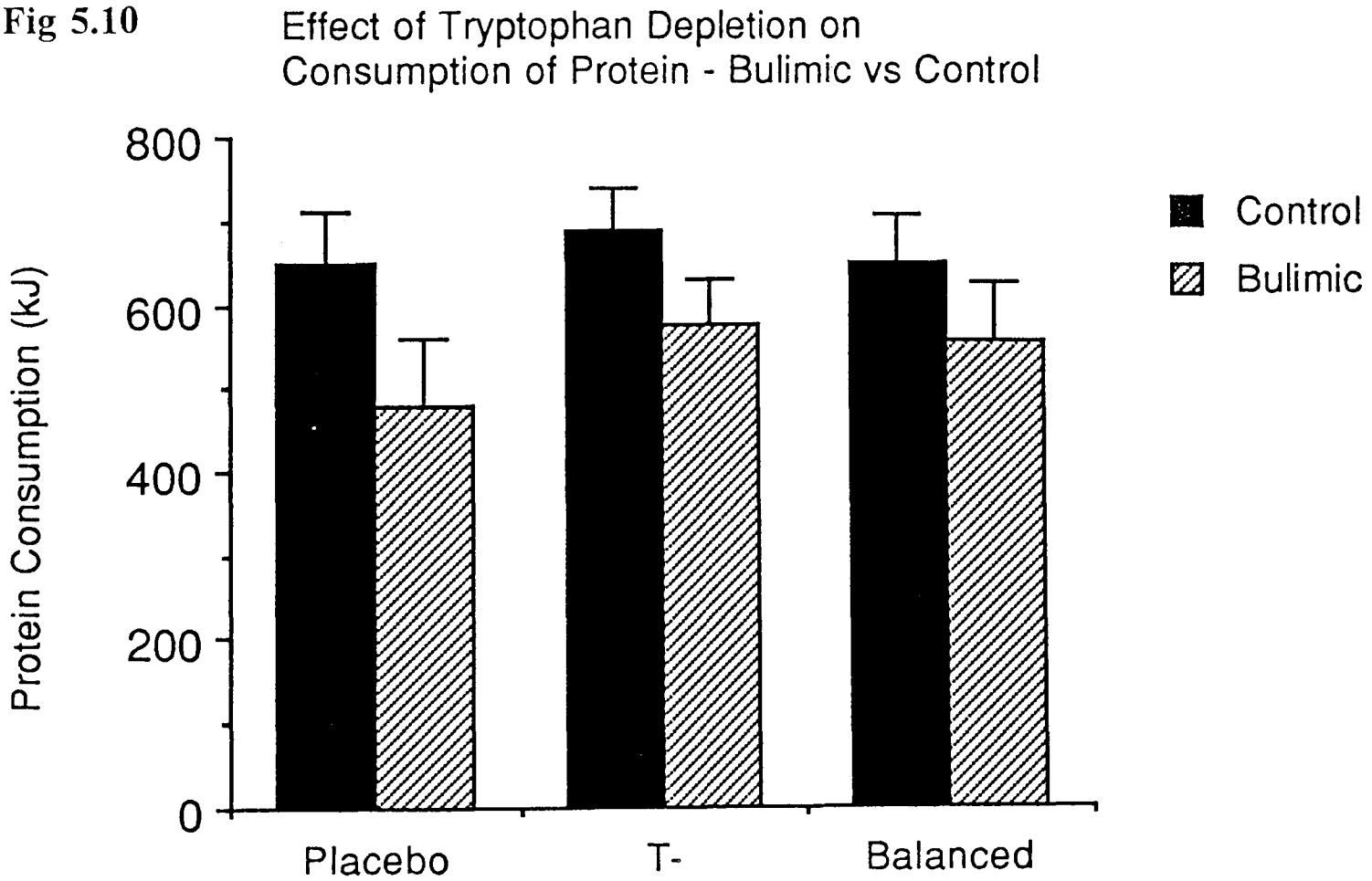
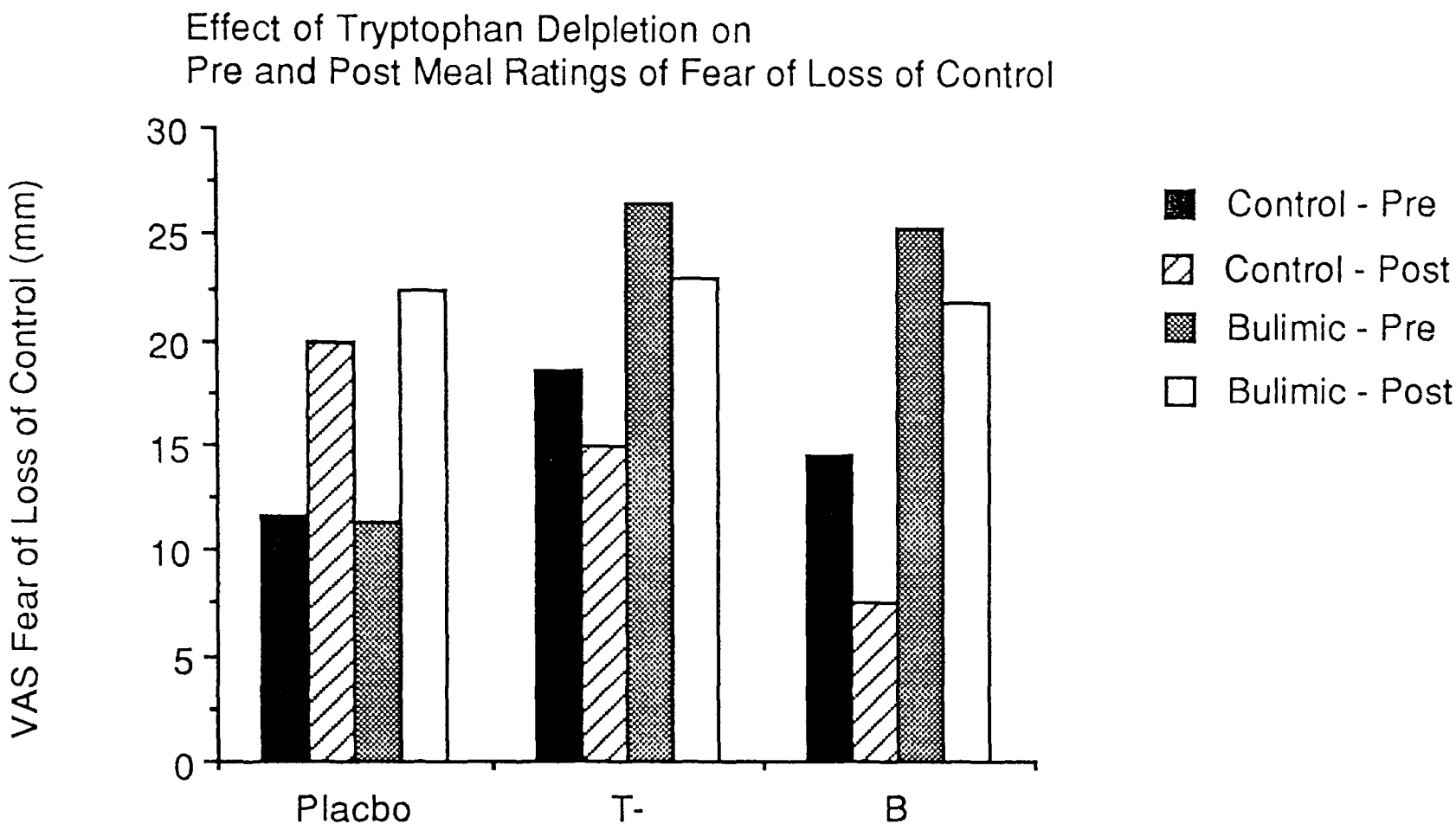


Fig 5.11


Effect of Tryptophan Delpletion on
Pre and Post Meal Ratings of Fear of Loss of Control
VAS Fear of Loss of Control (mm)
30
25
20
15
10
5
0
Placbo
T-
B
Control - Pre
Control - Post
Bulimic - Pre
Bulimic - Post

Mood Related Measures

Measures of mood were analyzed to examine the effects of tryptophan depletion on the lowering of mood. These were VAS ratings of 'despondency', 'irritability', 'tension' and 'anxiety', and POMS ratings of depression and anxiety/tension. The ANOVA findings for these measures are set out in Table 5.9.

For all measures of mood there was a significant main effect of group (Table 5.9). These effects came from the higher ratings of negative mood in the RBN group (Table 5.10). In addition, there was a significant effect of condition on POMS ratings of depression with depression ratings of both groups being higher on the initial placebo day (Table 5.11). However, there was no effect of time on ratings of mood, and no interactions between time, group and condition (all p values >0.1) (Fig 5.12a/b). These data indicate that the T- drink did not increase negative ratings of mood in either the RBN subjects or controls.

Analysis of Diary Ratings for Recovered Bulimic Group

In addition to the above measures, the RBN group was asked to complete a daily diary of mood and appetite for the week following each experimental session. This consisted of daily VAS ratings for mood and abnormal eating behaviour, a POMS rating for the first two days, and a BDI at the end of the week. It was expected that changes in appetite, as predicted above, would persist for longer than the experimental session, and these measures were designed to assess these changes. The results of these are set out below.

Fig 5.12a

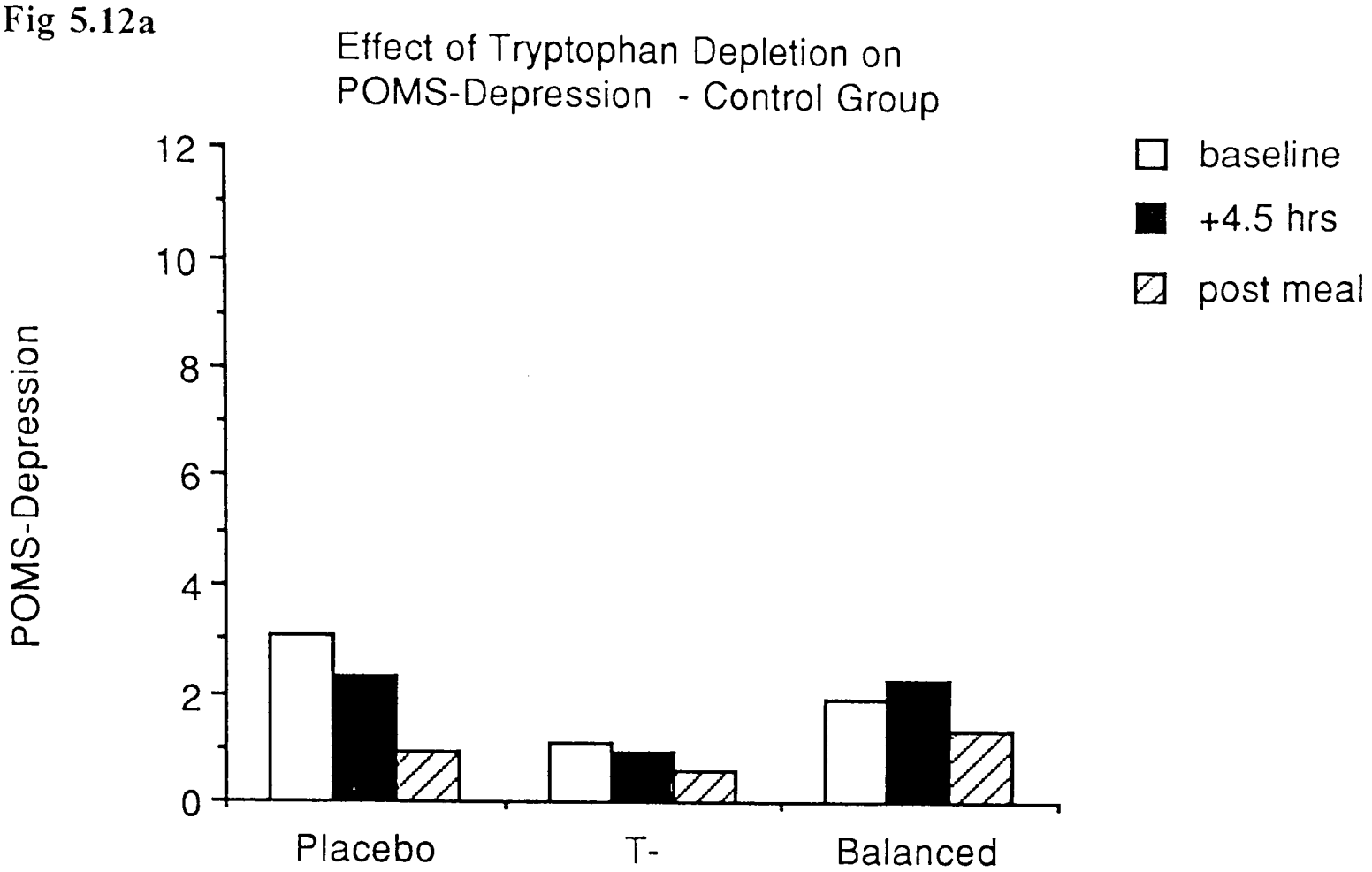
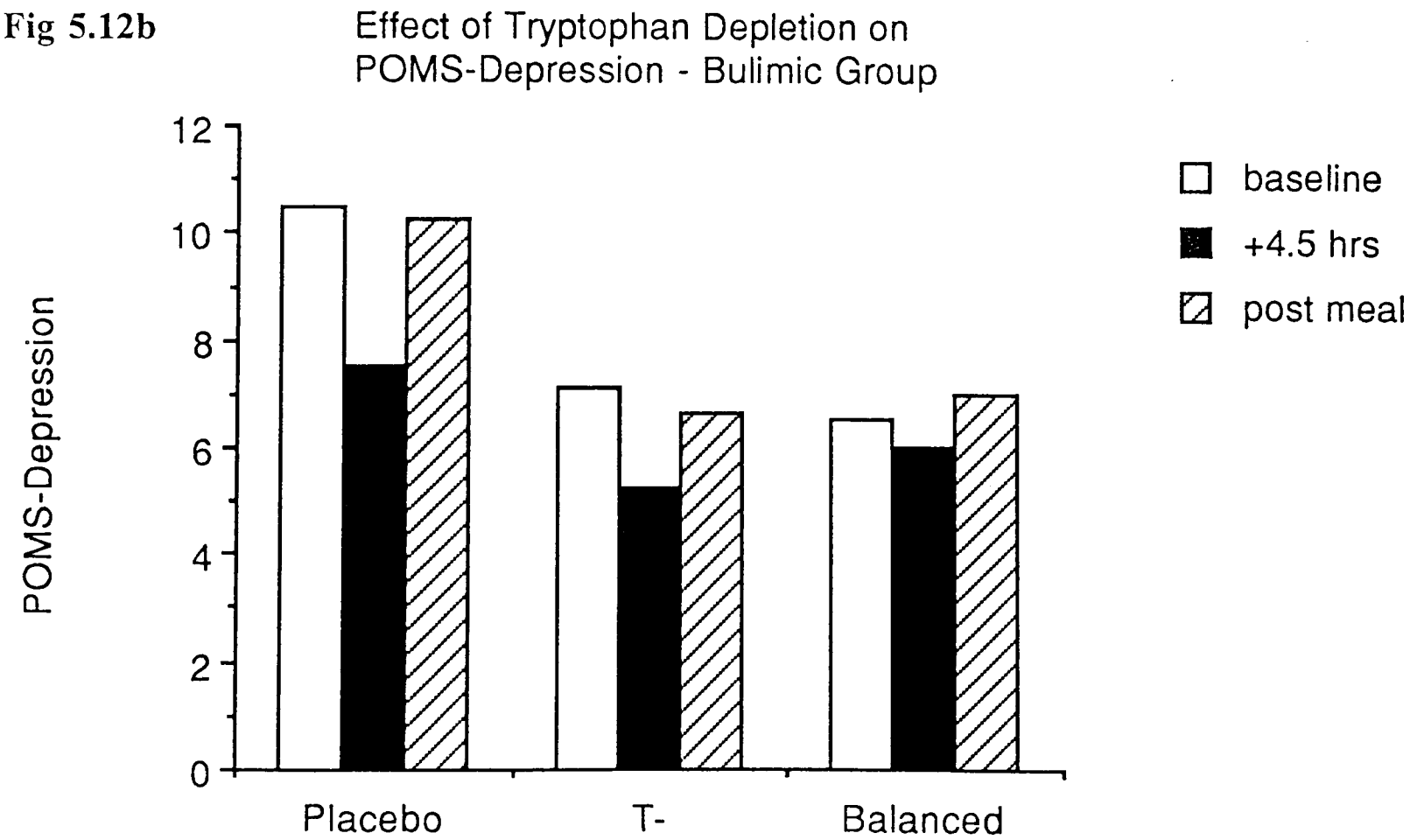


Fig 5.12b



Diary VAS, POMS and BDI ratings of mood. The ANOVA findings for these measures are set out in Table 5.12. For all VAS of mood and for the BDI ratings taken from the diaries, the analysis of variance revealed no significant main effects of condition or time, and no significant interactions (Table 5.13).

Analysis of POMS ratings for anxiety/tension revealed no significant main effect of condition or time, but a significant condition by time interaction (Table 5.13). This effect came from the baseline placebo condition, where subjects tended to rate themselves as more anxious than for the remaining two conditions. For POMS ratings of depression there were no significant main effects of condition or time, or condition by time interaction.

Eating Disturbance

The ANOVA findings for the VAS ratings of eating disturbance obtained from diary ratings are set out in Table 5.14. For these measures there were no significant main effects of condition or time, and no condition by time interactions. For fear of loss of control, there was a trend for a main effect of condition. This appeared to be coming from elevated scores in the balanced drink condition (Table 5.15).

5.4 DISCUSSION

Differences between recovered bulimic subjects and controls. There were a number of interesting differences between the RBN and control group. However, because of the small sample size in the RBN group, the present findings must be considered as preliminary. Despite the fact that the RBN subjects met strict criteria for recovery (several of them for many years), as a group they exhibited consistent evidence of disordered eating patterns and abnormal attitudes to food. This was consistent with differences seen in the test meal, where RBN subjects consumed significantly fewer calories than controls. In addition to these differences in appetite, the RBN group consistently reported higher levels of dysphoric mood.

Appetite. The RBN subjects rated themselves as having less wish to eat before meals than the controls despite no difference in ratings of hunger, and reported the meal to be less satisfying. The RBN subjects also felt that they could eat smaller amounts of food than the controls before the meal. This was reflected in their actual calorie consumption which was significantly less than the controls, but interestingly, they reported greater wish to eat after the meal than the controls. It would therefore seem that RBN subjects were exercising dietary restraint (decreased calorie consumption) in addition to cognitive restraint (they had a decreased wish to eat, and anticipated they could eat small amounts of food only) when presented with a meal.

This apparent restraint occurred despite the fact that none of the RBN subjects were underweight. Furthermore, they did not differ significantly on ratings of restraint (as assessed using the EDE-Q3).

The decrease in calorie intake in the RBN subjects was characterised by a decrease in fat consumption at test meal. The food preference questionnaires revealed that RBN subjects chose significantly fewer carbohydrate items when forced to select between carbohydrate and protein items. This is not inconsistent with the decrease in fat intake, as many of the carbohydrate items were also relatively high in fat. The reduction of calorie and fat consumption seen in the placebo condition (which was always the first test session) was presumably a result of the unfamiliarity of the laboratory setting.

Recovered bulimic subjects also reported significantly higher ratings on measures of disordered eating. Compared with controls, the RBN subjects were more concerned about overeating and losing control of their eating, concerns that are typical of patients who are actively bulimic.

There have been very few studies examining responses to meals in recovered bulimic populations. However, one study, using diary assessment, reports that recovered bulimics consume significantly **larger** meals (increased calorie, carbohydrate and protein consumption) than controls (Elmore and de Castro, 1991). Their meal patterns were reported as being similar to active bulimic patients. However, the criteria for recovery in this study were inadequate for proper interpretation of the data; volunteers were assessed by questionnaire which categorised them as recovered on the basis of bingeing and purging behaviour only. The present study adopted a full clinical interview in conjunction with measures of restraint, attitudes to body weight and shape, and eating attitudes.

Mood. The elevated ratings of negative mood in the RBN subjects were seen on several measures; recovered bulimics rated themselves as more depressed, tense, anxious and

irritable than the controls. To date, studies exploring the recovery process in bulimia nervosa have limited themselves to an analysis of symptoms associated with disordered eating (Johnson-Sabine et al, 1992; Herzog et al, 1993). This study therefore highlights an important area for further study. Whilst it is well recognised that bulimia nervosa is associated with affective disorder, the precise nature of this association is poorly understood (see section 1.3.2.2 for review). Clearly, the negative mood experienced by the RBN subjects in the present study was not clinical in nature (BDI scores were not significantly elevated in this group), but it would be important to investigate further the precise nature of negative mood in recovered bulimic patients. Furthermore, longitudinal studies may elucidate whether there is an underlying mood disorder in individuals who develop bulimia nervosa, thus furthering our understanding of the causal relationship between the two disorders.

Biochemical measures. An unexpected but consistent finding was the reduction in baseline plasma total tryptophan levels found in the recovered bulimic subjects. This finding is of interest in light of the evidence that brain 5-HT function is impaired in patients with bulimia nervosa. Whilst in actively bulimic patients there does not appear to be a reduction in plasma tryptophan (Lydiard et al, 1988), it is possible that the metabolic abnormalities associated with active bulimia might obscure a pre-existing deficit in plasma tryptophan concentration.

Chapter 3 of the present thesis demonstrated that dieting in normal female subjects is associated with a reduction in plasma total tryptophan. If dietary restriction demonstrated by the recovered bulimic subjects in the test-meal paradigm is representative of their usual dietary habits, the changes in plasma tryptophan seen in the present study could be

attributable to a chronic reduction in food intake. Clearly, further studies are needed in order to assess whether this deficit in plasma tryptophan is associated with restrained eating, or whether it is an enduring condition. The effects of chronic dietary restraint on plasma tryptophan are poorly understood, and investigations carried out on normal populations would clearly be of interest. Furthermore, the relationship between full recovery from bulimia nervosa and eating behaviour are also poorly understood, and further documentation of this would shed light on the course of recovery, and possible intervention strategies.

Effects of tryptophan depletion. As found with the previous study, the 50g T- drink led to substantial reductions in plasma total and free tryptophan. However, despite the increased vulnerability of the recovered bulimic subjects to low mood, tryptophan depletion had no effect on mood. This was surprising, as several of the subjects had personal and family histories of depression.

There was also no effect of tryptophan depletion on measures of appetite. It is possible that the overnight and morning fast may have produced a ceiling effect on hunger and food intake which obscured any effects of tryptophan depletion. However, Goodall and Silverstone (1988) found in a similar experimental paradigm that food intake in healthy subjects could be increased by the 5-HT receptor antagonist, metergoline. However, with the recovered bulimic subjects in particular, it is possible that the laboratory setting proved rather intimidating, and the food consumed at test meal was not a true reflection of their eating patterns. It has been demonstrated that reliable data on eating behaviour can be collected in active bulimics (Kaye et al, 1988). This involves a laboratory adapted to be as naturalistic as possible, a facility that was not available for the present study.

The hypothesis that tryptophan depletion would be associated with temporary symptomatic relapse in the recovered bulimic subjects was not supported by the data. In many respects the recovered bulimic subjects tested could be considered a vulnerable group. For example they had elevated pre-treatment dysphoria scores on the POMS scale, and evidence of fears of overeating and loss of control. In addition, their plasma tryptophan levels were lowered prior to testing, suggesting some degree of compromise of brain 5-HT pathways even before tryptophan depletion.

It is possible that the 50g dose of amino acids was too low to provoke clinical symptoms in the recovered bulimic subjects. Studies reporting relapse in depressed patients adopted a 100g dose (Delgado et al, 1990). Alternatively, a more chronic depletion of tryptophan may be necessary to provoke relapse, although the data from this study appear to suggest that recovered bulimic subjects are in an enduring state of relative tryptophan deficiency even when they are clinically recovered. This implies that if decreased tryptophan availability is important in the development of bulimia nervosa, its pathophysiological effects must be exerted through interaction with other factors.

In conclusion, this study suggests that recovered bulimic subjects may have persistently abnormal eating attitudes and behaviour. This is in keeping with the finding that whilst the antidepressant, imipramine, suppressed binge frequency in active bulimic patients, food intake outside binges remained depressed (Rossiter et al, 1988). They also appear to be in an enduring state of tryptophan deficiency, but are not vulnerable to the effects of tryptophan depletion.

Together with the previous studies, the data suggest that lowering plasma tryptophan is not

associated with changes in appetite. There may be several reasons for this lack of effect. First, it is experimentally harder to demonstrate increases in appetite than appetite suppression (see section 1.5.2.3). An alternative approach to the study of the mechanisms underlying hyperphagia involves administration of an anorectic compound, and examining the effect of potentially hyperphagic compounds on this experimentally induced anorexia (eg Goodall and Silverstone, 1988). This overcomes the problem of ceiling effects which may be responsible for the lack of effect in the current and previous study. This approach will be addressed in Chapter 7.

Methodologies adopting a manipulation of general 5-HT depletion or enhancement have elucidated the importance of this neurotransmitter in the control of appetite. However, with the growing identification of specific receptor subtypes of the 5-HT neurone, there has been a growing interest in the potential role for these in the control of appetite. Whilst this issue has not been fully addressed by human studies, preliminary animal work suggests that there may be a role for 5-HT_2 and 5-HT_{1A} in the control of appetite (see section 1.5.2.3 for review). It is therefore clearly of theoretical importance to address these findings in relation to the regulation of human appetite. The remaining two chapters of the present thesis will therefore concentrate more specifically on receptor subtype function in relation to appetite.

CHAPTER 6 THE EFFECTS OF META-CHLOROPHENYLPIPERAZINE ON NEUROENDOCRINE RESPONSES AND FOOD INTAKE IN HEALTHY FEMALE VOLUNTEERS

6.1 INTRODUCTION

There has recently been a growing literature on the role of 5-HT receptor subtypes in the control of feeding in animals. This work has concentrated mainly on the suppression of food intake via enhancement of brain 5-HT function with compounds that are selective or partially selective for specific receptor subtypes (see section 1.5.2.3 for review).

In vitro studies of the rat have demonstrated that mCPP binds to 5-HT_{2C}, 5-HT_{2A}, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_3 receptors (Hamik and Peroutka, 1989; Hoyer 1988b; Kilpatrick et al, 1987). However, its binding affinity is most potent at the 5-HT_{2C} receptor (Hoyer, 1988b). mCPP also has some binding affinity to α_1-, α_2-, and β-adrenergic receptors (Kahn and Wetzler, 1991). In humans, evidence suggests that neuroendocrine effects of mCPP are specific to stimulation of the 5-HT system. For example, the 5-HT_1/5-HT_2 antagonist, metergoline, blocks mCPP-induced prolactin, cortisol and ACTH release (Mueller et al, 1986; Kahn et al, 1990a).

Human studies have shown mCPP to be a reliable 5-HT agonist which can be used to study brain 5-HT function in humans (Kahn and Wetzler, 1991). Studies using oral doses have produced reliable endocrine responses characterised by an increase in cortisol and prolactin (see Kahn and Wetzler, 1991 for review). Neuroendocrine and behavioural findings from

intravenously administered mCPP are not as well defined as the effects of oral administration. For studies using oral dosing, data are available on the serum half-life of mCPP. For example, with 0.5mg/kg, the half-life was approximately three hours (Murphy et al, 1989).

Administration of mCPP is also associated with a number of physical symptoms. High oral doses (eg 0.5mg/kg) have been associated with sweating, light-headedness, palpitations, anxiety and nausea (Kahn et al, 1990b). However, the available data suggest that lower doses (eg 0.25mg/kg, orally) are not generally associated with these symptoms (Kahn et al, 1990b).

The physiological responses to mCPP have been examined to some extent. Some studies report an elevation of body temperature in response to oral and iv administration (Murphy et al, 1989; Mueller et al, 1985), although there are some negative findings (Kahn et al, 1990). mCPP appears to increase blood pressure and, in some circumstances, pulse rate, although these effects are less consistent than those seen for temperature (see Kahn and Wetzler, 1991 for review).

It has been demonstrated that administration of mCPP consistently decreases food intake in rats (Samanin et al, 1979; Fuller et al, 1981; Kennet and Curzon, 1988). Furthermore, studies using selective 5-HT antagonists to attenuate the hypophagic effect of mCPP suggest that this effect appears to be mediated via activation of 5-HT_{2C} receptors (Kennett and Curzon, 1991). This hypophagic effect does not appear to be a result of decreased locomotor activity (Dourish, 1992), and is not associated with nausea or general malaise as pre-treatment with an antiemetic does not alter the effect (Kennett and Curzon, 1988).

A recent study in humans reported that the anorectic effect of d-fenfluramine was attenuated by the selective 5-$HT_{2A/2C}$ antagonist, ritanserin, suggesting that the 5-HT_{2C} receptor may be involved in regulating food intake in humans (Goodall et al, 1993). There are, however, no published reports of the effects of mCPP on appetite in humans.

The present study therefore aims to assess the effect of mCPP on appetite, plasma PRL and oral temperature in healthy female volunteers. Low doses were chosen for this investigation in order to minimise occurrence of symptoms such as nausea which would confound alterations in appetite. Oral doses of 7.5mg and 15mg were therefore compared with placebo. These doses have been shown to decrease slow wave sleep, suggesting that they are sufficient to activate brain 5-HT_{2C} receptors (Katsuda et al, 1994).

As discussed in section 1.5.2.3, there are interesting theoretical reasons why it is important to study mechanisms controlling appetite in females in particular. For example, repeated exposure to stress (known to increase central 5-HT in the rat (Dunn and Welch, 1991)) is associated with a persistent reduction in food intake in female rats only (Kennett et al, 1986; Haleem et al, 1988). There are clear sex differences in fenfluramine and RU2469-induced hypophagia in the rat, with greater hypophagia seen in females (Rowland et al, 1986). Furthermore, of particular interest to the present chapter, administration of mCPP is associated with a similar effect, although this is only seen in 24-hour food-deprived animals (Haleem, 1988; Clifton et al, 1993). Whilst the underlying mechanisms controlling this effect remain unclear, these findings are of potential theoretical significance in furthering our understanding of how eating behaviour may become dysregulated more readily in female populations. Whilst the present study did not aim to compare directly the responses of males and females to mCPP, it was felt that a hypophagic response to mCPP

in females would be of greater clinical significance. It was hypothesised that mCPP would be associated with a decrease in food intake at test meal and decrease in subjective ratings of hunger and appetite, in addition to increases in plasma PRL and oral temperature.

6.2 METHODS

Volunteers

Twelve healthy female volunteers were recruited through advertisement to take part in a study looking at the effects of mCPP on mood and appetite. Volunteers were carefully screened to ensure that they were in good health, had no history of psychiatric disorder or eating difficulties, and no current dietary restrictions. All volunteers were within the normal range of weight for height (mean BMI=22.5, SD=1.9, range=19.5-25.5), and were within the normal range of eating attitudes as assessed by the Eating Attitudes Test (EAT) (Garner and Garfinkel, 1979); (mean EAT=6, SD=3.02, range=1-11). Current levels of depression were assessed using the Beck Depression Inventory (BDI) (Beck et al, 1961). All volunteers fell within the normal range (mean=2.6, SD=2.1, range=0-6). The age of volunteers fell within the range of 22 to 37 years (mean=29, SD=4.7). All women had a regular 28 day menstrual cycle.

Experimental Design

The design was a double-blind, placebo controlled cross-over with a balanced order of drug administration. Subjects were tested on three occasions receiving a) mCPP, 7.5mg; b) mCPP, 15mg; c) placebo. All medication was given in identical capsules. Experimental session fell within days 3-16 of the menstrual cycle (ie end of menstruation/follicular). Tests were carried out at least five days apart, which meant that the study extended over two consecutive menstrual cycles.

Procedure

Assessment. Assessment and screening procedures were the same as those followed in the diet study (see Chapter 3 for assessment protocol).

Experimental Sessions. Subjects arrived at the research unit at 8.30am. They had been instructed to eat normally for the previous day (ie follow their normal eating patterns), and to fast from midnight. Volunteers rested quietly on a bed in a neuroendocrine testing room for the whole of the morning until lunchtime. At 8.30am (-30mins), an indwelling venous cannula was inserted, and 30mins later mCPP was administered. Further blood samples were then taken at 30min intervals for the following three hours. Oral temperature was taken at 30min intervals throughout the test, using a glass-mercury thermometer which remained in situ for 10mins before the reading was taken. The test meal was presented three and a half hours after ingestion of mCPP (see Chapter 2 for protocol).

Subjects were reimbursed the sum of £45 for successful completion of the study.

Measures

Mood. During the test sessions, mood was measured using 100mm anchored visual analogue scales (VAS). The parameters were happy, light-headed, anxious, and despondent/sad (Appendix 6.1). POMS ratings (McNair et al, 1971) were taken at baseline, pre- and post-meal; VAS ratings were taken at 30min intervals across the morning, and post-meal.

Appetite. Appetite was measured using the free choice, buffet-style test meal accompanied by VAS and food preference questionnaires (as described in Chapter 2). In addition, 100mm anchored VAS scales were used (as for mood). The parameters were hungry and wish to eat (Appendix 6.1). Levels of nausea were measured in the same way (Appendix 6.1).

Biochemical Measures

Plasma was separated immediately after collection by centrifugation and stored at -30°C. Plasma prolactin (PRL) was determined by a standard immunoradiometric assay. Inter- and intra-assay coefficients of variation over the range encompassed by the standard curve were 7.0% and 3.3% respectively. The measurement of mCPP in plasma was carried out using high performance liquid chromatography as described by Franklin (1992).

Data Analysis

The endocrine, temperature and VAS data were analyzed with a two-way repeated measures analysis of variance (ANOVA), with post-hoc t-tests where the interaction term was significant. Data from the test meal were analyzed with a one-way repeated measures ANOVA. The endocrine, temperature and plasma levels of mCPP were also measured as Area Under the Curve (AUC) using the trapezoid measure. These summary measures were compared using Wilcoxon's signed rank test. Correlations were carried out using Pearson's product moment. Data were analyzed using SPSS for PC (version 6.0) and OXSTAT.

6.3 RESULTS

Prolactin and temperature. Following placebo, plasma PRL levels tended to fall to a plateau after about two hours. Compared to placebo, mCPP increased plasma PRL (Fig 6.1). The ANOVA showed a significant main effect of time ($F_{5,55}$=2.77, $p<0.05$), of drug ($F_{2,22}$=4.54, $p<0.05$), and a significant drug by time interaction ($F_{10,110}$=7.45, $p<0.001$). Means and standard deviations are presented in Table 6.2. Post-hoc t-tests revealed that PRL levels were significantly higher following mCPP than they were following placebo. Plasma PRL levels tended to be higher following the 15mg dose of mCPP compared to 7.5mg, but this difference was not significant (Fig 6.1). The AUC of PRL response was significantly higher following mCPP than after placebo for both the 7.5mg ($p<0.05$) and 15mg ($p<0.01$) doses. These were not significantly different from each other.

Oral temperature was higher following mCPP compared to placebo. Means and standard deviations are presented in Table 6.2. The ANOVA showed a significant main effect of time ($F_{5,55}$=9.75, $p<0.001$), and of drug ($F_{2,22}$=4.42, $p<0.05$), but the drug by time interaction was not significant ($F_{10,110}$=1.68, $p>0.05$) (Fig 6.2). The AUC temperature response was significantly higher following 15mg of mCPP than after placebo ($p<0.005$) but not after 7.5mg. The AUC temperature response did not differ significantly between the two doses of mCPP.

Appetite. Total calories consumed at test meal were not altered by mCPP ($F_{2,22}$=1.54, $p>0.05$) (Fig 6.3). Similarly there was no significant alteration in intake of any of the macronutrients: consumption of protein $F_{2,22}$=1.1, $p>0.05$; carbohydrate $F_{2,22}$=1.8, $p>0.05$;

Fig 6.1

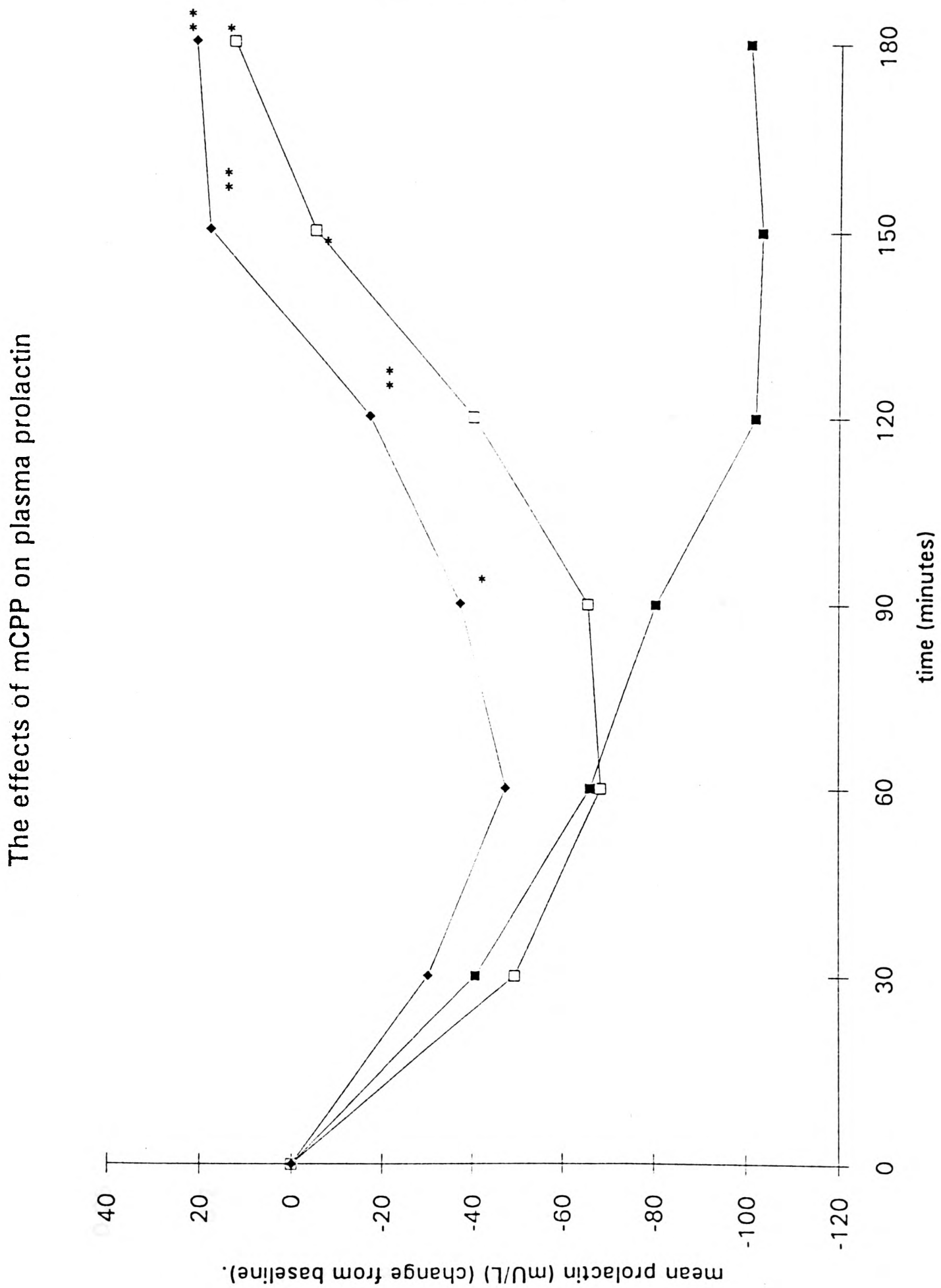

The effects of mCPP on plasma prolactin
placebo
low
high
* p<0.05, ** p<0.05, significantly different from placebo
mean prolactin (mU/L) (change from baseline).
40
20
0
-20
-40
-60
-80
-100
-120
0
30
60
90
120
150
180
time (minutes)

Fig 6.2

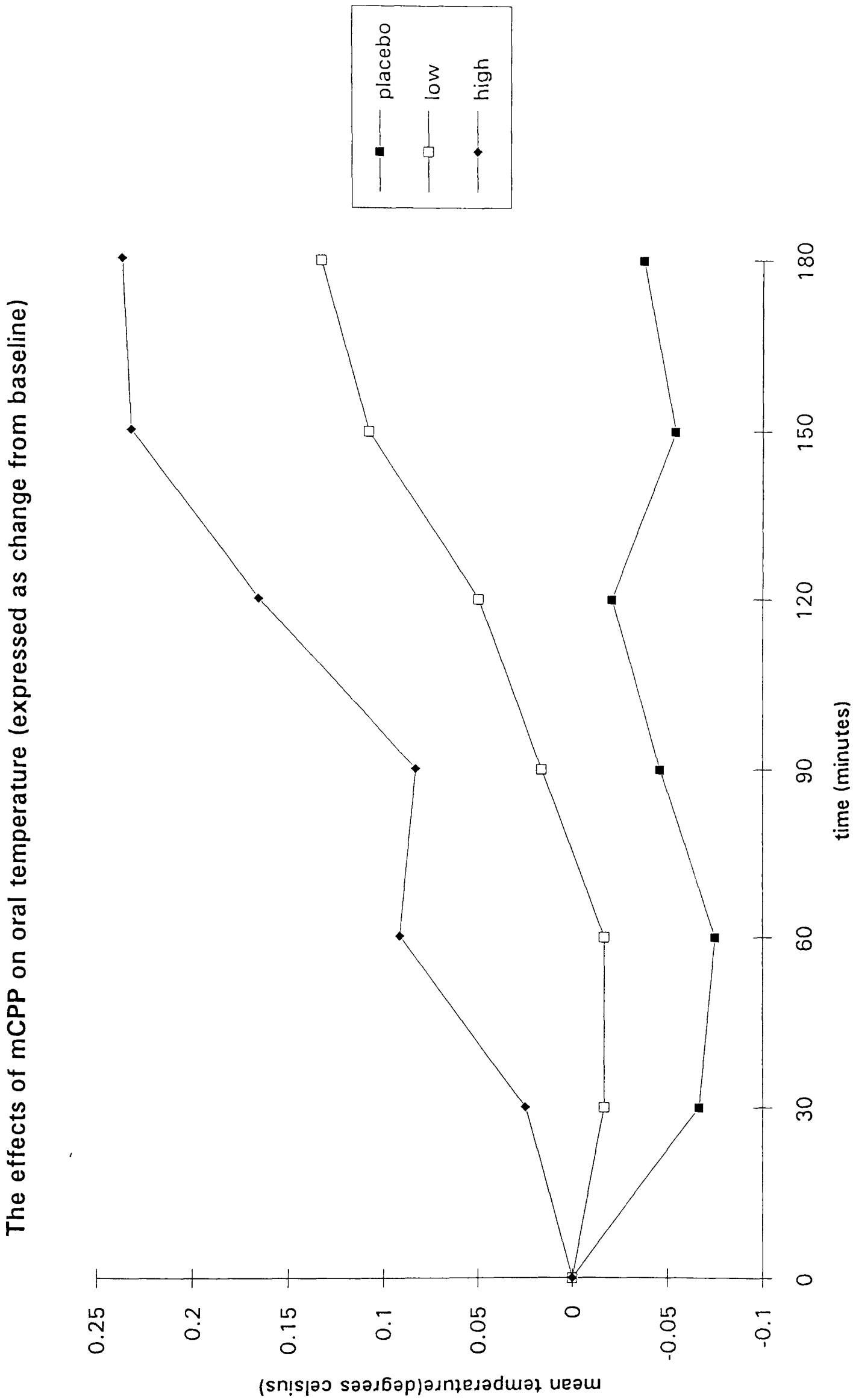

The effects of mCPP on oral temperature (expressed as change from baseline)
placebo
low
high
mean temperature(degrees celsius)
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
0
30
60
90
120
150
180
time (minutes)

Fig 6.3 The Effects of mCPP on Total Calorie Consumption at Test Meal

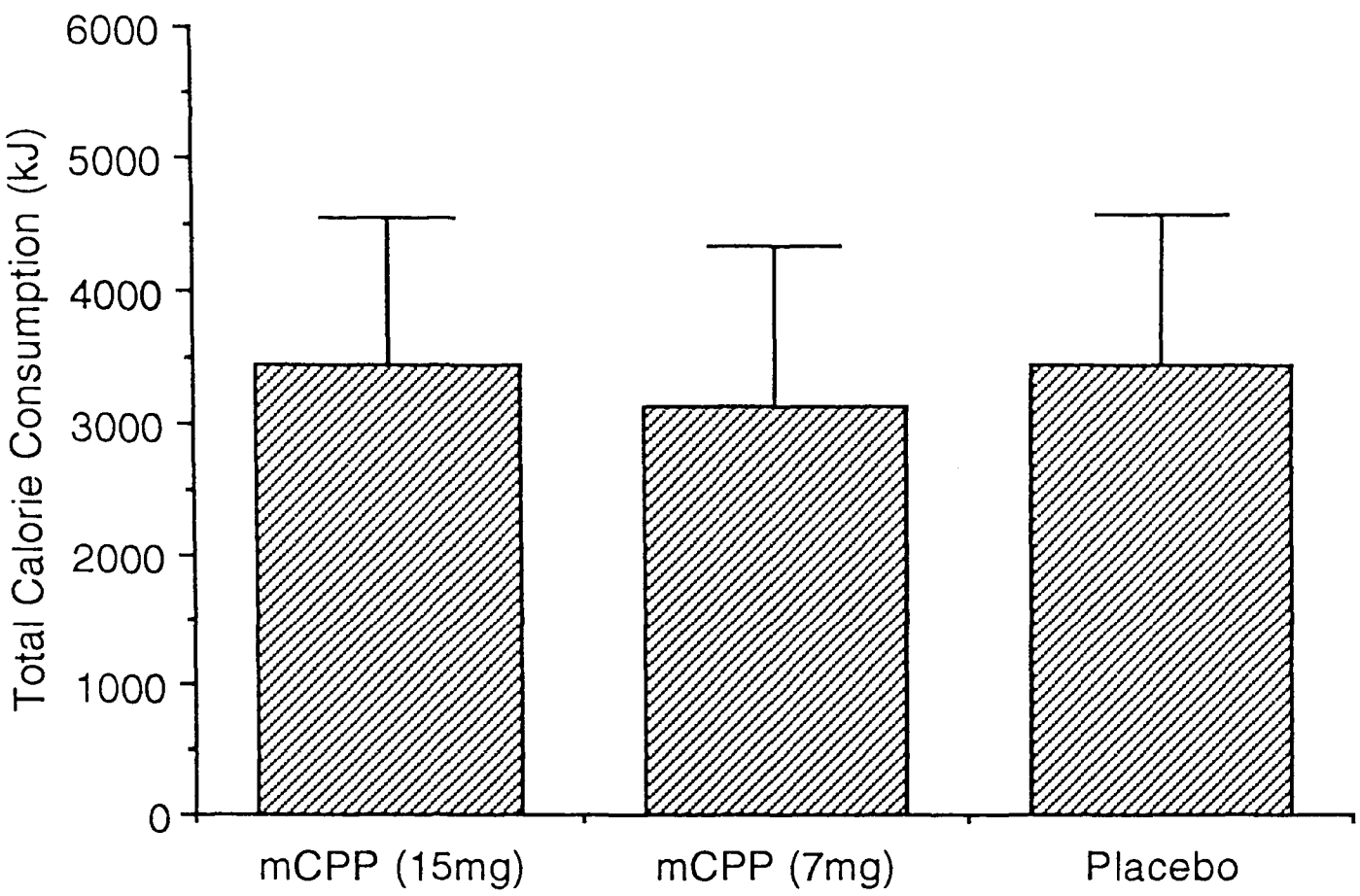
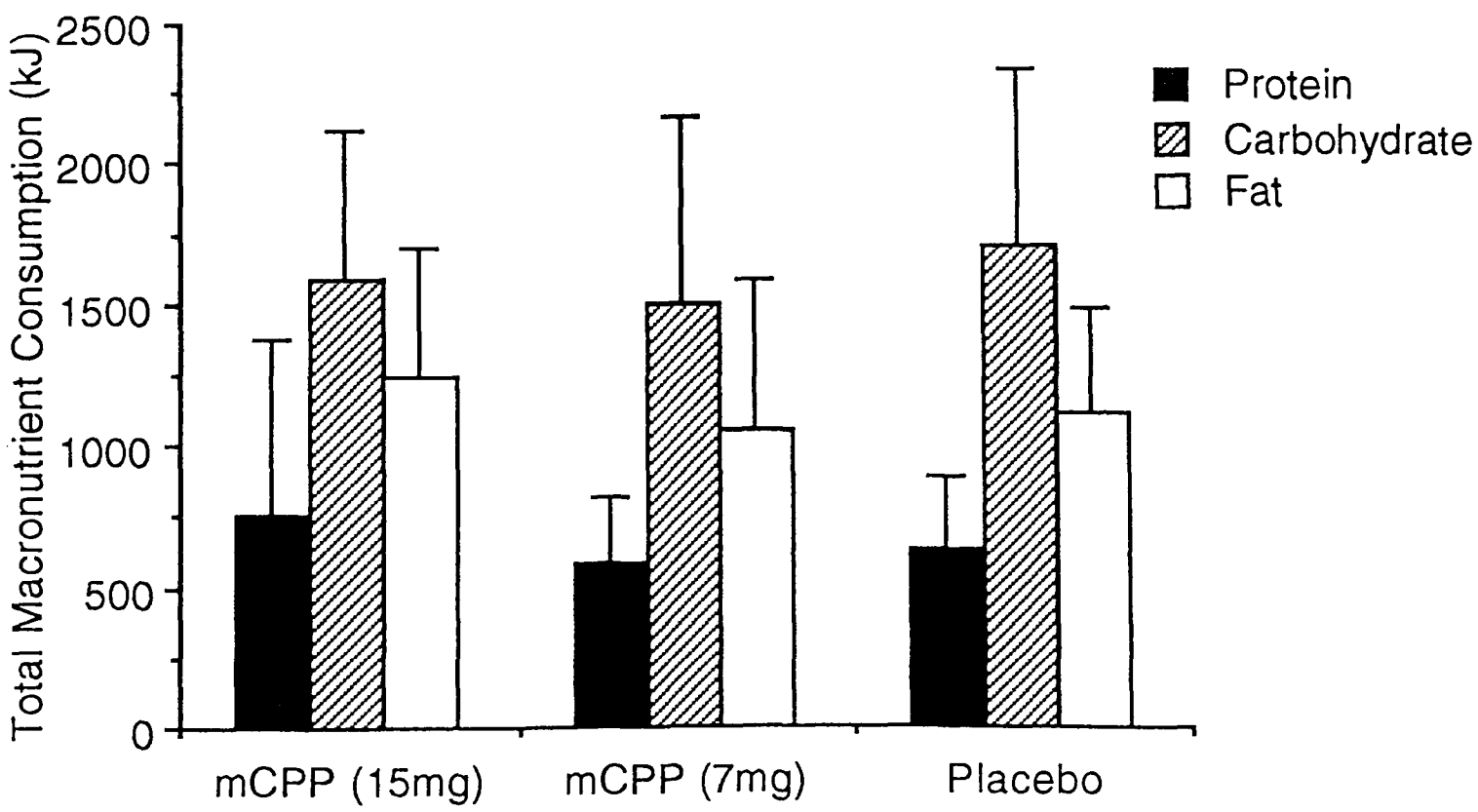


Fig 6.4 The Effects of mCPP on Macronutrient Selection at Test Meal



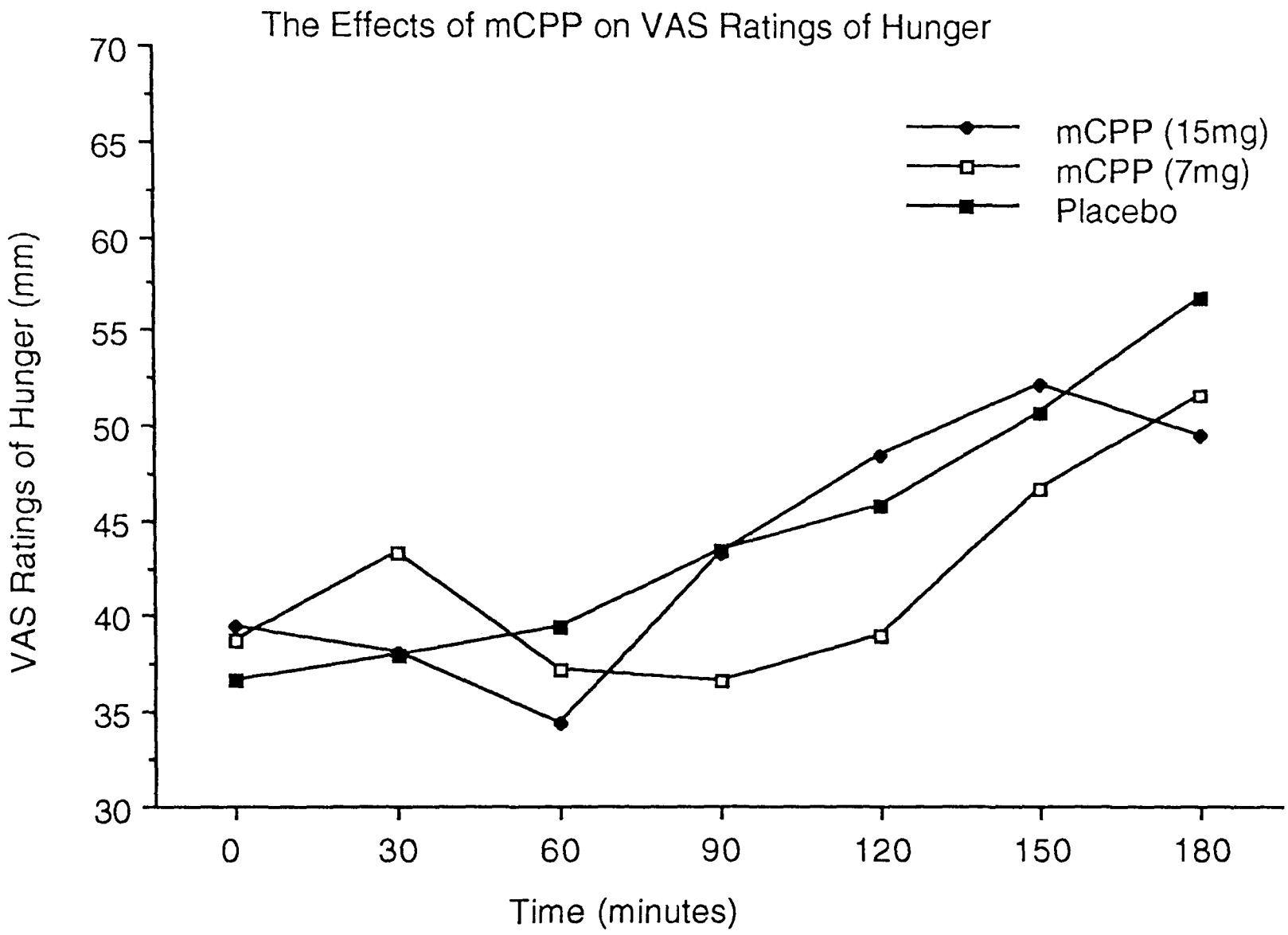
and fat $F_{2,22}$=1.64, p>0.05) (Fig 6.4). Means and standard deviations are set out in Table 6.3.

For VAS ratings of 'hunger' (Fig 6.5) and 'desire to eat', there was a significant main effect of time as these ratings increased across the morning ($F_{6,66}$=12.18, p<0.001 and $F_{6,66}$=8.50, p<0.001 respectively). There were, however, no main effects of drug ($F_{2,22}$=1.06, p>0.05 and $F_{2,22}$=2.62, p>0.05), and no drug by time interactions on these measures ($F_{12,132}$=1.49, p>0.05 and $F_{12,132}$=0.42, p>0.05 respectively). Means and standard deviations for 'hunger' are set out in Table 6.4.

Analyses of variance were carried out for pre- and post-meal VAS ratings of meal satisfaction, feelings of fullness, and desire to eat. As expected, the test meal significantly increased feelings of fullness ($F_{1,10}$=139.9, p<0.001), decreased desire to eat ($F_{1,10}$=98.0, p<0.001), and was associated with higher levels of satisfaction than pre-meal expectations ($F_{1,10}$=8.55, p<0.05). There were, however, no main effects of time, and no drug by time interactions on these measures (all F's <1). Means and standard deviations are set out in Table 6.7.

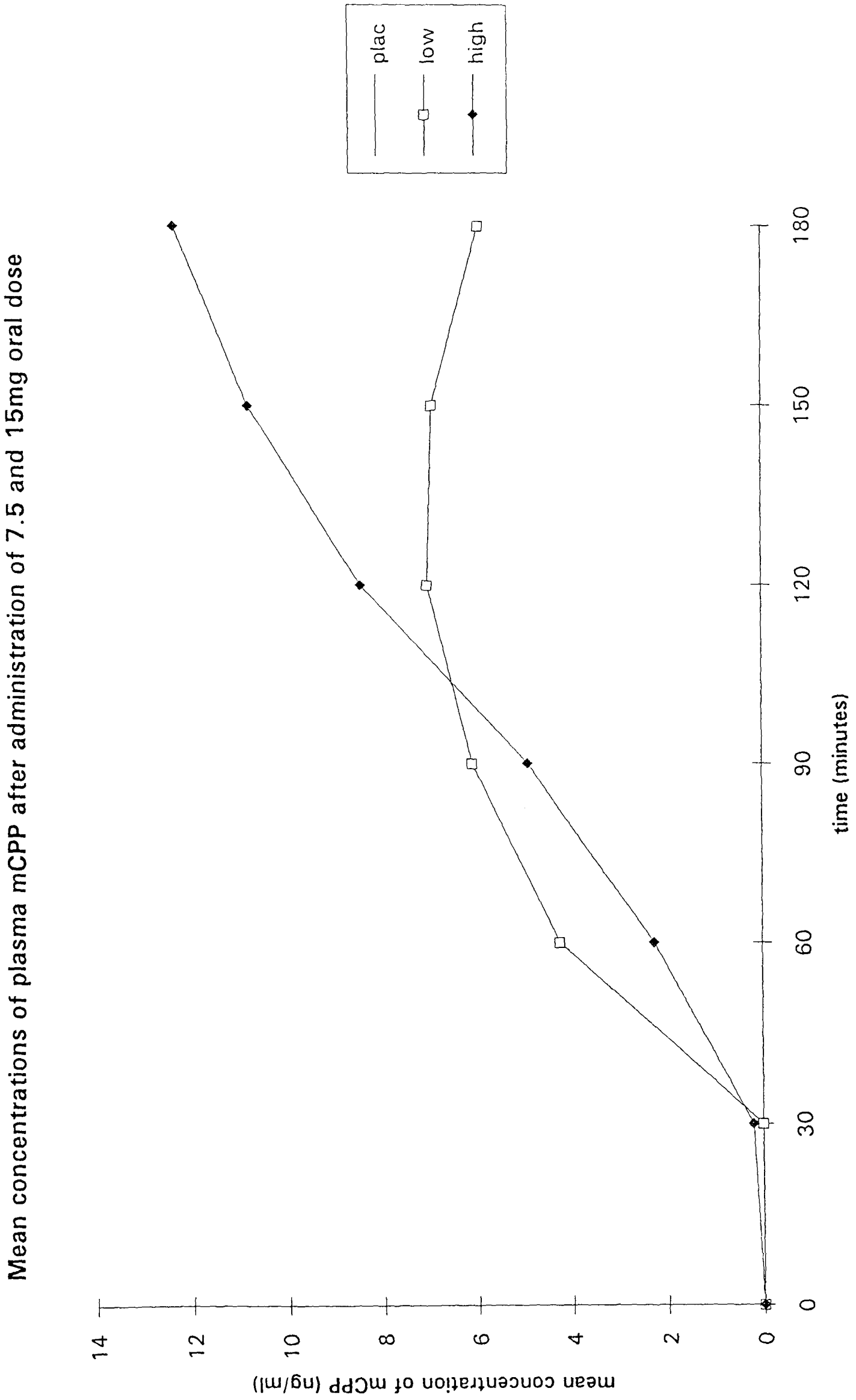
Mood. Analyses of variance for these measures are set out in Table 6.8. VAS ratings of 'light-headed' were increased by mCPP ($F_{2,22}$=4.03, p<0.05), but there was no main effect of time ($F_{6,66}$=1.33, p<0.05), and no interaction of drug by time ($F_{12,132}$=0.85, p>0.05). mCPP did not alter ratings of 'nausea', 'anxiety', 'happy' or 'despondent/sad'. Means and standard deviations for 'anxiety' and 'nausea' are set out in Tables 6.5 and 6.6.

Fig 6.5


The Effects of mCPP on VAS Ratings of Hunger
VAS Ratings of Hunger (mm)
70
65
60
55
50
45
40
35
30
0
30
60
90
120
150
180
Time (minutes)
mCPP (15mg)
mCPP (7mg)
Placebo

Plasma mCPP levels and correlations. Plasma mCPP could be detected in plasma about one hour after dosing. Levels of mCPP after the 15mg dose appeared to be higher than after the 7.5mg dose and were still slowly rising at the end of the test (Fig 6.6). However, the ANOVA showed no significant drug by time interaction ($F_{5,55}$=1.87, p>0.05), and the AUC of plasma mCPP was not significantly higher after the 15mg dose than after the 7.5mg dose. There was a significant correlation between plasma mCPP levels following the 15mg dose of mCPP and the AUC of plasma PRL (r=0.65; p=0.02) but not after the 7.5mg dose (r=0.38; p=0.21). There was no significant correlation between plasma mCPP levels and temperature response at either dose (p>0.1). There was a significant negative correlation between plasma mCPP levels after the 7.5mg dose and total food intake (r=-0.168; p=0.032) and borderline negative correlation between these measures after the 15mg dose of mCPP (r=-0.55; p=0.061). Means and standard deviations are set out in Table 6.9.

Fig 6.6


Mean concentrations of plasma mCPP after administration of 7.5 and 15mg oral dose
plac
low
high
mean concentration of mCPP (ng/ml)
0
2
4
6
8
10
12
14
time (minutes)
0
30
60
90
120
150
180

6.3 DISCUSSION

The findings presented here confirm that mCPP increases plasma PRL and oral temperature in healthy volunteers. Furthermore, the effect on plasma PRL was apparent at doses lower than those employed in previous studies. There was, however, no clear dose-related response, suggesting that these lower doses may lie at the lower end of the dose response curve, or that the difference in plasma levels of mCPP between the two doses was too small to have an effect on plasma PRL. The relative lack of subjective effects of mCPP at these doses in healthy volunteers is in keeping with previous investigations (Kahn and Wetzler, 1991).

Animal studies suggest that, with sufficient dosage, mCPP has a clear hypophagic effect (Samanin et al, 1979; Fuller et al, 1981; Kennet and Curzon, 1988). It was therefore surprising that the present study found no significant effect of mCPP on total calorie consumption or macronutrient consumption at test meal. Furthermore, there was no effect on subjective ratings of hunger and appetite either across the morning, or in response to the test meal. One possible explanation for this is that the doses of mCPP employed were too low to produce a measurable anorectic effect. These doses were selected on the basis that they are well tolerated, and appear to activate brain 5-HT_{2C} receptors as measured by alterations in slow wave sleep in humans (Katsuda et al, 1993, Sharpley et al, 1994). The finding that plasma levels of mCPP correlated inversely with food intake suggests that larger doses may produce a significant anorectic effect. It is also possible that because of the overnight and morning fast, subjects were sufficiently hungry to produce a ceiling effect of intake at test meal. However, this seems unlikely as previous studies demonstrating anorectic effects have employed overnight fasts (Silverstone et al, 1980; Goodall and

Silverstone, 1988). It may be that subsequent meal patterns and snacks could be modified by mCPP, and a study adopting a longer period of observation would be of interest.

A further explanation for this lack of effect may be that the animal model of mCPP-induced hypophagia is not applicable to humans. Animal studies often adopt a longer period of prior food deprivation (typically 24 hours), and with mCPP this is associated with potent decrease in food intake (Samanin et al, 1979; Kennett and Curzon, 1988). Alternatively, mCPP may not act as an anorectic agent in humans; it has been argued that the anorectic effect of mCPP in animals requires activation of the 5-HT_{1B} receptor in addition to the 5-HT_{2C} receptor (Kennett and Curzon, 1988). While humans possess a homologue of the 5-HT_{1B} receptor, the pharmacological characteristics of this receptor are virtually the same as the human 5-HT_{1D} receptor (Oksenberg et al, 1992), for which mCPP has a rather low affinity (Hoyer et al, 1988b). If the anorectic action of mCPP does, in fact, require simultaneous activation of 5-HT_{1B} and 5-HT_{2C} receptors, this effect may not be readily achievable in humans. Furthermore, as mCPP has some affinity to the α_2-adrenergic receptor in addition to the 5-HT receptors, it is possible that stimulation of the NA system may be important in the effects of this drug on appetite. It has been proposed, for example, that amphetamine-induced anorexia is mediated by NA release (Silverstone et al, 1980, Blundell and Latham, 1980). It seems unlikely, however, that activation of the NA system induced by mCPP would act in a similar fashion; evidence to date suggests that amphetamine anorexia is associated with activation of the β-adrenergic receptor (Sanghvi et al, 1975; Goodall et al, 1987) to which mCPP binds weakly. However, the mechanisms underlying amphetamine anorexia remain unclear, and it is possible that activation of α-adrenergic receptors may be important (Silverstone and Kyriakides, 1982). Furthermore, unpublished data (cited in Kahn, 1991) suggest that oral and iv doses of mCPP do not

elevate blood levels of 3-methoxy,4-hydroxyphenylglycol (MPHG), the central metabolite of NA, suggesting that the behavioural and physiological effects of mCPP are not mediated via activation of the NA system.

However, a recent study adopting the same basic design, but a different dosing regime found an anorectic response to mCPP (Walsh et al, pers comm). This study examined the effects of 0.4mg/kg of mCPP on food intake in healthy female volunteers, and found a striking decrease in food intake and hunger ratings. The most likely explanation for this effect is the increased dose, which is equivalent to approximately 20-25mg. This initial finding suggests that mCPP does have anorectic properties in humans, although it remains unclear whether this is secondary to the effects of mCPP on symptoms such as nausea. Whilst Walsh et al (pers comm) found no effect of mCPP on nausea prior to test meal, there had been an initial increase in this measure in the earlier part of the test morning. Further research would be needed in order to establish the possible impact of this on appetite, but the data do suggest that the 5-HT_{2C} receptor may be important in the control of appetite in humans.

Clearly, it would be of interest to pursue the potential of mCPP as an anorectic agent by assessing the effects of longer term administration on appetite. Such a study would be of great clinical interest in the treatment of eating disorders, especially obesity. Alternatively, an understanding of the mechanisms underlying the action of known anorectic compounds is of considerable theoretical importance with regard to clinical intervention. These drugs fall broadly into two classes: amphetamine and amphetamine derivatives, and drugs which enhance 5-HT neurotransmission (eg fenfluramine, fluoxetine). Whilst much animal work has been carried out to this end (Sanghvi et al, 1975; Blundell and Latham, 1980; Neill and

Cooper, 1989; Gibson et al, 1993), the precise mechanisms underlying the anorectic properties of these drugs remain largely unexplored in humans. Recently a series of human experiments have shown that the action of d-fenfluramine is likely to be mediated via the 5-HT_2 receptor family (Goodall and Silverstone, 1988; Goodall et al, 1993). However, the role of 5-HT in amphetamine anorexia is less clearly understood, although preliminary animal evidence suggests that the 5-HT_3 receptor subtype may have a role (Cooper et al, 1993). This has been further supported by one human study (Silverstone et al, 1992), and will be pursued in the next chapter of the present thesis.

CHAPTER 7 THE EFFECTS OF GR68755 ON AMPHETAMINE ANOREXIA IN HEALTHY VOLUNTEERS

7.1 INTRODUCTION

A wealth of evidence exists for the hypophagic effect of amphetamine both in animals and humans (Garattini and Samanin, 1976; Silverstone and Stunkard, 1968; Blundell and Latham, 1978). The dextro-rotary isomer d-amphetamine is particularly potent, and reduces food intake in a dose related manner (Silverstone and Stunkard, 1968). Doses greater than 10mg are sufficient to decrease appetite in healthy volunteers (Silverstone, 1985). Much research has centred on which central neurotransmitters mediate this effect; studies in animals have demonstrated that amphetamine increases the release and inhibits reuptake of NA and DA (Carlsson, 1970; Blundell, 1985). There appears also to be an increase in the release of 5-HT, particularly within the lateral hypothalamus (Hernandez et al, 1987; Hoebel et al, 1989; Parada et al, 1992).

To date, research suggests that amphetamine anorexia may be largely mediated by NA, although DA may also play a role; this might be either via direct appetite suppressing action or due to alterations in arousal and/or reward seeking behaviour (see section 1.5.2.2 for review). Whilst the specific role of DA in the control of food intake remains unclear, studies have examined the role of DA in the mediation of hedonic responses to food. Neuroleptic compounds have been shown to increase consumption of sweet, palatable foods (Xenakis and Sclafani, 1981), and are associated with weight gain in psychiatric populations (Harris and Eth, 1981), suggesting that DA may mediate feeding behaviour by a process of positive effects associated with consumption of these food types (Morley and Blundell,

1988). It is therefore possible that amphetamine acts in part to suppress food intake by altering the rewarding properties of food.

There is evidence to suggest that the 5-HT_3 receptor can modulate DA neurotransmission in the mesolimbic system (Costall et al, 1990). These receptors are particularly abundant in terminal areas of central DA pathways (Kilpatrick et al, 1987), and it is thought that 5-HT_3 receptor agonists increase mesolimbic dopaminergic neurotransmission (Blandina et al, 1988; Jaing et al, 1990). Blockade of 5-HT_3 receptors does not reduce basal output of DA or its metabolites (Carboni et al, 1989a), but attenuates the DA release induced by certain drugs such as nicotine and opiates (Imperato et al, 1989).

Behavioural studies provide further evidence for the role of 5-HT_3 blockade in the inhibition of DA related behaviours such as locomotor hyperactivity (see Montgomery et al, 1993, for review). It could be argued, therefore, that antagonism of 5-HT_3 receptors would attenuate amphetamine-induced DA release, thus attenuating the anorectic effect of amphetamine. To date, only one animal study has explored the effect of 5-HT_3 blockade on amphetamine anorexia; Cooper et al (1993) demonstrated that ondansetron augmented this effect in non-food-deprived rats. The authors offer no real explanation for this surprising result, but the data do confirm that 5-HT plays a role in amphetamine anorexia. However, results of a pilot study in human volunteers showed that ondansetron significantly attenuates the effect of amphetamine on subjective ratings of hunger at +2.5 hours after drug administration (Silverstone et al, 1992). This finding is in keeping with most of the animal literature reviewed above.

Research suggests that 5-HT_3 antagonists exert no independent effect on appetite and food intake (see Dourish, 1992 for review). However, these drugs are reported to increase gastric emptying in certain species, an effect which is often associated with hyperphagia (Duggan and Booth, 1986).

The aim of the present study is to explore further the effect of 5-HT_3 blockade on amphetamine anorexia. The novel compound GR68755 was employed to test this hypothesis. GR68755 is a second generation 5-HT_3 receptor antagonist which has been shown to inhibit locomotor hyperactivity in the rat caused by continuous infusion of DA into the nucleus accumbens, and by amphetamine injected into the nucleus accumbens (see Johnson, 1994). GR68755 is safe and well tolerated in healthy volunteers at doses studied (up to 16mg bd orally for 3.5 days, and 2mg orally for 27.5 days).

In humans a single intravenous dose of 2mg GR68755 reverses the amnesic effects of scopolamine at +1 and +3 hours after dosing. In addition, a single oral dose of GR68755 has been shown to antagonise 5-HT-mediated intradermal flare response, with a duration of action of almost ten hours (Johnson, 1994). For the purposes of this study a dose of 2mg was therefore selected. GR68755 is absorbed rapidly after oral dosing, with a short elimination half-life of 1.6 to 2.7 hours. Peak absorption is at 45 mins to 1 hour.

From previous research (see above), it was hypothesised that GR68755 would attenuate the amphetamine-induced decrease in appetite ratings across the test morning, and attenuate amphetamine-induced hypophagia at test meal.

7.2 METHODS

Volunteers

26 healthy male volunteers between the ages of 18 and 45 years (mean 28.9) were recruited to take part in a study looking at the psychological effects of amphetamine and GR68755. Volunteers were screened for history of psychiatric or major physical illness, and a general screening was carried out to ensure that they were in good physical health. Written informed consent was provided by the volunteers and their GP prior to entry into the study.

Experimental Design

The four treatments were administered in a randomized, double-blind, Latin square design: a) d-amphetamine (20mg oral) plus placebo GR68755; b) placebo GR68755 and placebo d-amphetamine; c) GR68755 (2mg oral) plus placebo amphetamine; d) GR68755 plus d-amphetamine.

Each experimental session was separated by 4-7 days. Prior to testing, all subjects were familiarized with the test room conditions.

Measures

Appetite. Appetite was measured following the protocol set out in section 2.2, using the buffet style test meal, VAS's and food preference questionnaires.

Procedure

Subjects arrived in the unit at 0800 hours having fasted from 2330 hours the previous night. A specimen of urine was taken for analysis of drugs, and subjects then rested quietly in the test room for a 30 minute period. At 0830 hours, baseline VAS ratings were completed. At 0900 hours, GR68755 or matching placebo was administered, and thirty minutes later d-amphetamine or matching placebo was given. VAS ratings were completed throughout the morning every 20 minutes until 1230 hours (3 hours in total). At 1230 hours, subjects completed the test meal protocol as set out in section 2.2. This consisted of pre-meal rating scales (VAS's, free and forced choice food preference questionnaires), followed by the test meal, and post-meal ratings (Appendices 2.6-2.9).

Data Analysis

Where data were repeated measures, they were analyzed by three-way analysis of variance (ANOVA) (Amph by GR68755 by Time) using the SPSS-PC statistical package, version 6.0. The remaining data were analyzed by two-way analysis of variance (Amph by GR68755). These analyses included a total of 22 subjects; 2 subjects were excluded from the analysis as they were found to be positive on the drug screen, and a further 2 did not complete all 4 test sessions.

7.3 RESULTS

The ANOVA revealed a highly significant main effect of amphetamine for VAS ratings of hunger ($F_{1,21}$=33.8, p<0.0001), but not of GR68755 ($F_{1,21}$=2.47, p>0.05) (Fig 7.1). There was also no significant interaction of amphetamine by GR68755 ($F_{1,21}$=0.21, p>0.05); hunger was equally suppressed in both conditions. There was a significant main effect of time ($F_{1,21}$=4.49, p<0.0001), and this interacted significantly with both drug conditions ($F_{1,21}$=14.93, p<0.0001 for amphetamine; $F_{1,21}$=2.71, p<0.005 for GR68755), suggesting that amphetamine attenuated the increase in hunger across the morning. There was however, no significant three-way interaction for hunger ($F_{1,21}$=0.78, p>0.05). (Table 7.1).

For 'desire to eat', the ANOVA findings followed a similar pattern to those seen with hunger. There was a significant main effect of amphetamine ($F_{1,21}$=21.6, p<0.0001), but not of GR68755 ($F_{1,21}$=1.62, p>0.05), and no significant interaction of amphetamine by GR68755 ($F_{1,21}$=0.17, p>0.05) (Fig 7.2). This came from the suppression of desire to eat by amphetamine in a similar fashion to its effects on hunger. With desire to eat there was also a significant main effect of time ($F_{1,21}$=4.5, p<0.0001), and this interacted significantly with both drug conditions ($F_{1,21}$=16.16, p<0.0001 for amphetamine; $F_{1,21}$=2.48 p<0.01 for GR68755), suggesting that amphetamine attenuated the increase in desire to eat across the morning. Similarly to hunger, there was no significant three-way interaction for desire to eat ($F_{1,21}$=1.28, p>0.05). (Table 7.2).

Analysis of variance was also carried out on post meal VAS ratings of satiety in order to examine the effects of amphetamine and GR68755. These measures were 'found meal satisfying' and 'feel full' (see Table 7.3 for means and SD). For satisfaction of meal there

Fig 7.1

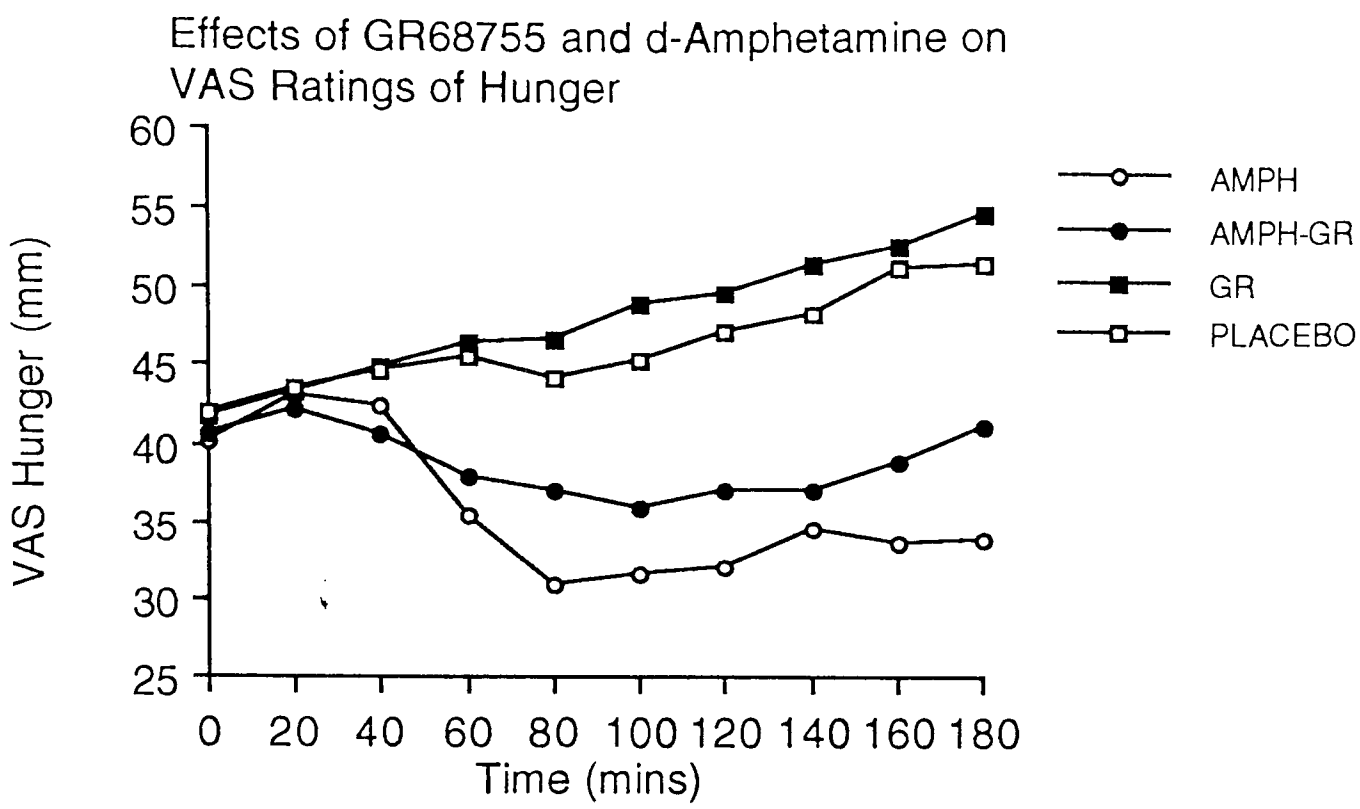
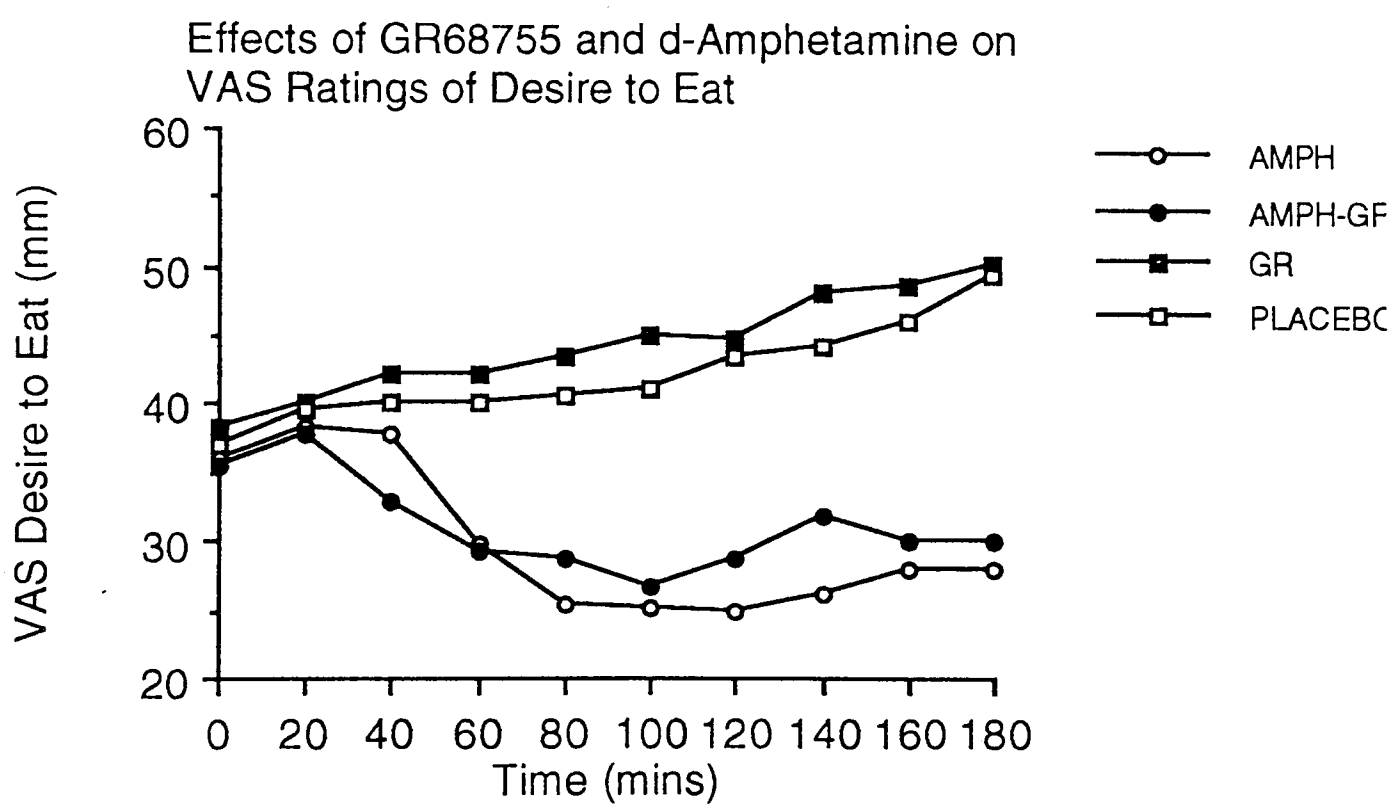

Effects of GR68755 and d-Amphetamine on
VAS Ratings of Hunger
VAS Hunger (mm)
60
55
50
45
40
35
30
25
0 20 40 60 80 100 120 140 160 180
Time (mins)
AMPH
AMPH-GR
GR
PLACEBO

Fig 7.2


Effects of GR68755 and d-Amphetamine on
VAS Ratings of Desire to Eat
VAS Desire to Eat (mm)
60
50
40
30
20
0 20 40 60 80 100 120 140 160 180
Time (mins)
AMPH
AMPH-GF
GR
PLACEBC

was a significant main effect of amphetamine ($F_{1,21}$=11.8, p=<0.01), but not of GR68755 ($F_{1,21}$=1.62, p>0.05). This came from the decrease in satisfaction ratings seen with amphetamine. There was no significant interaction of amphetamine by GR68755 ($F_{1,21}$=0.66, p>0.05).

Similarly, for feelings of fullness there was a significant main effect of amphetamine ($F_{1,21}$=9.8, p<0.01), but not of GR68755 ($F_{1,21}$=1.43, p>0.05). This was due to decreased ratings of fullness in the amphetamine condition alone. There was no significant interaction of amphetamine by GR68755 ($F_{1,21}$=0.6, p>0.05).

For calorie intake at test meal, the ANOVA revealed a significant main effect of amphetamine for total calorie intake ($F_{1,21}$=21.28, p<0.001), but not for GR68755 ($F_{1,21}$=0.4, p>0.05) (Fig 7.3). There was no amphetamine by GR68755 interaction ($F_{1,21}$=0.67, p>0.05). These results were a reflection of the suppression of food intake seen in the amphetamine condition. (See Table 7.4 for means and SD).

This pattern of suppression of intake by amphetamine held true for analysis of macronutrient content of test meals. For protein there was a significant main effect of amphetamine ($F_{1,21}$=16.86, p<0.001), but not of GR68755 ($F_{1,21}$=0.17, p>0.05), and no drug interaction ($F_{1,21}$=0.66, p>0.05) (Fig 7.4). Similarly, for carbohydrate intake there was a significant main effect of amphetamine ($F_{1,21}$=17.29, p<0.001), but not of GR68755 ($F_{1,21}$=0.28, p>0.05), with no drug interaction ($F_{1,21}$=0.8, p>0.05) (Fig 7.5). For fat intake there was also a significant main effect of amphetamine ($F_{1,21}$=22.97, p<0.001), but not of GR68755 ($F_{1,21}$=0.5, p>0.05), with no drug interaction ($F_{1,21}$=2.82, p>0.05) (Fig 7.6). (See Table 7.4 for means and SD).

Fig 7.3

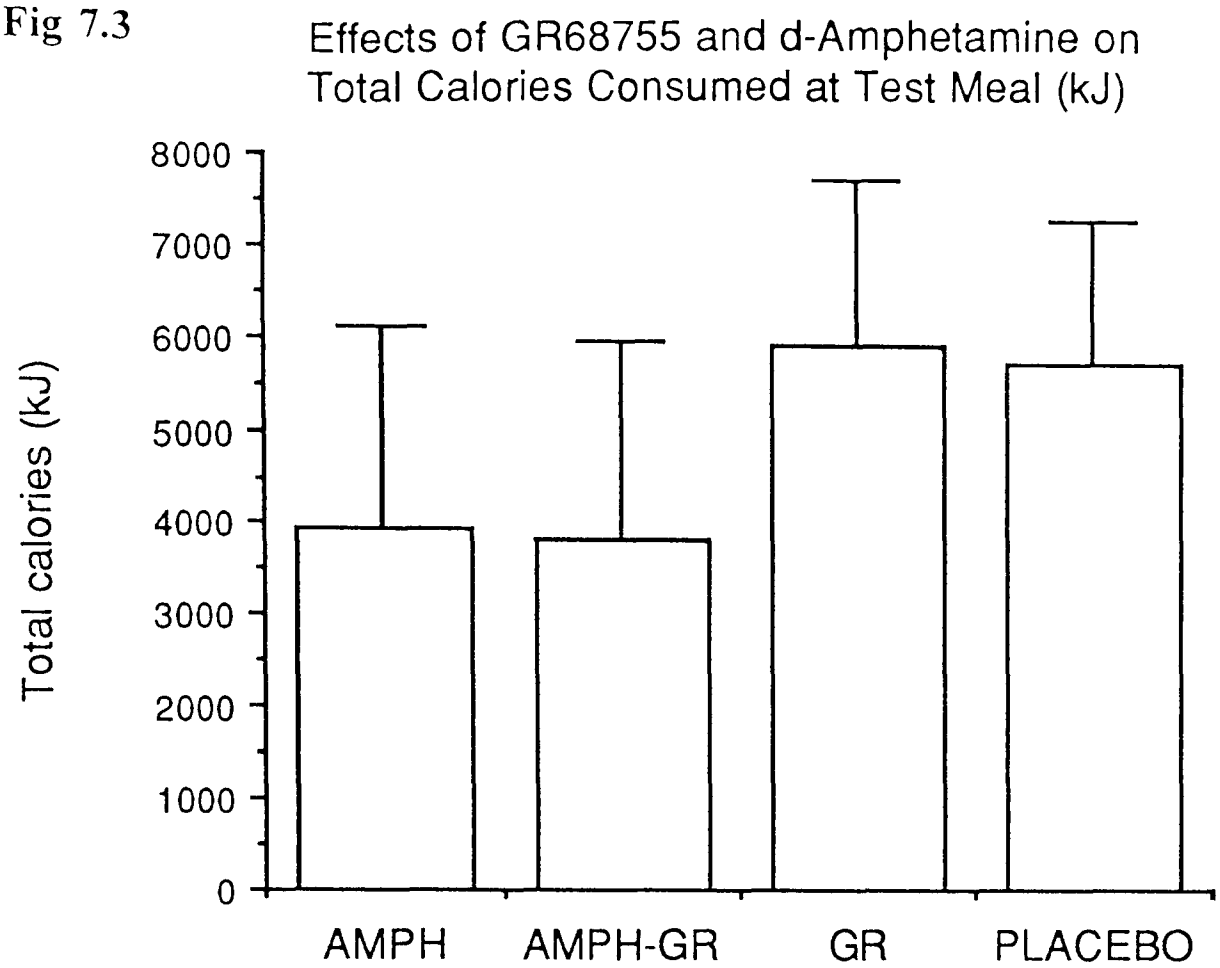

Effects of GR68755 and d-Amphetamine on
Total Calories Consumed at Test Meal (kJ)
Total calories (kJ)
8000
7000
6000
5000
4000
3000
2000
1000
0
AMPH
AMPH-GR
GR
PLACEBO

Fig 7.4

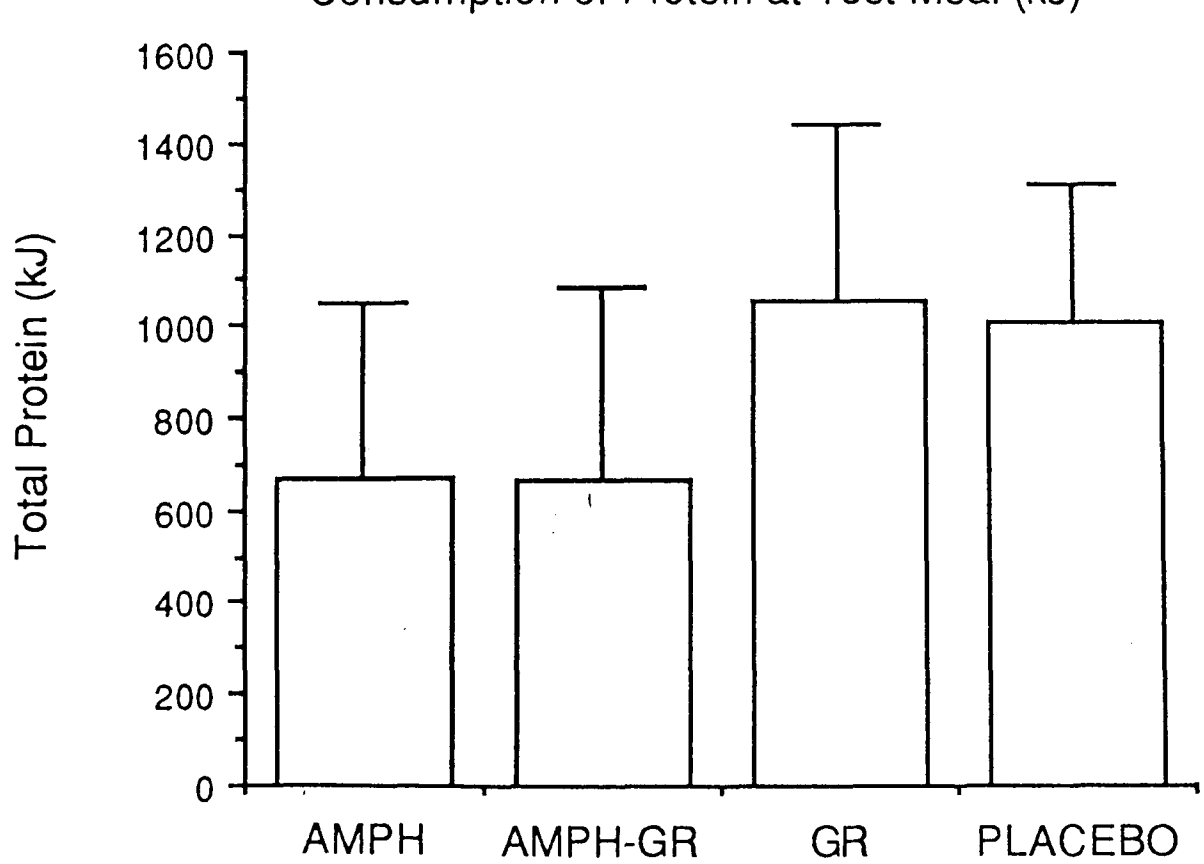

Effect of GR68755 and d-Amphetamine on
Consumption of Protein at Test Meal (kJ)
Total Protein (kJ)
1600
1400
1200
1000
800
600
400
200
0
AMPH
AMPH-GR
GR
PLACEBO

Fig 7.5

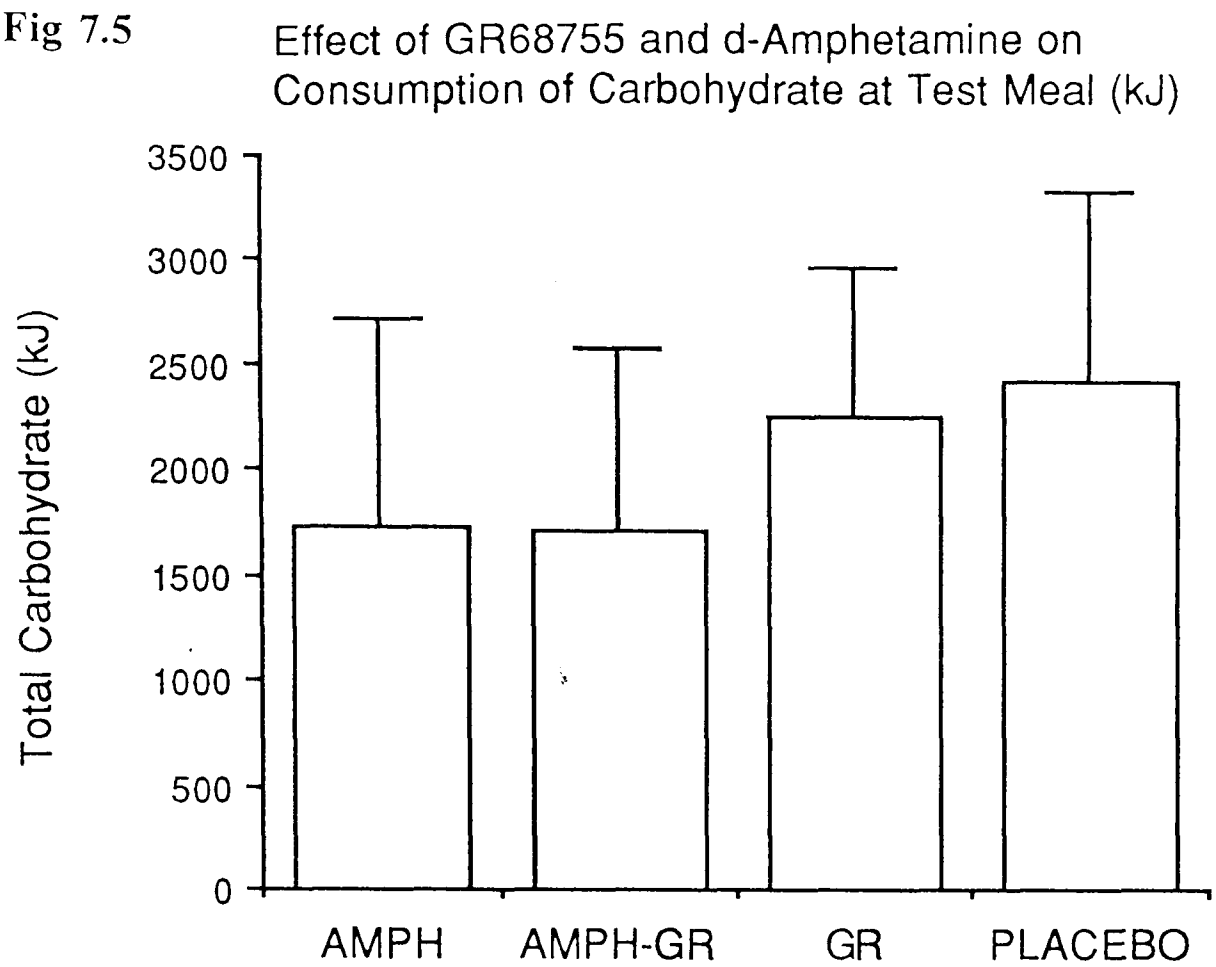
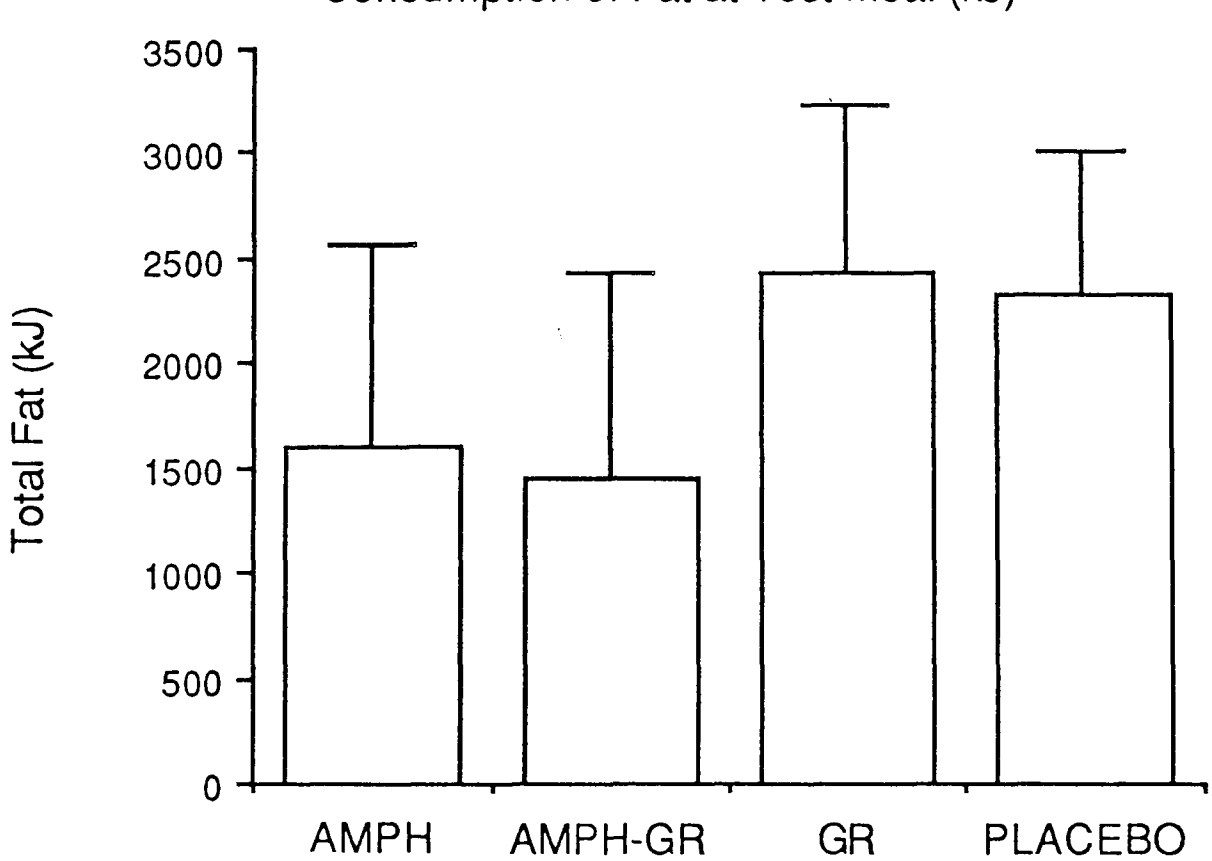

Effect of GR68755 and d-Amphetamine on
Consumption of Carbohydrate at Test Meal (kJ)
Total Carbohydrate (kJ)
3500
3000
2500
2000
1500
1000
500
0
AMPH
AMPH-GR
GR
PLACEBO

Fig 7.6


Effect of GR68755 and d-Amphetamine on
Consumption of Fat at Test Meal (kJ)
Total Fat (kJ)
3500
3000
2500
2000
1500
1000
500
0
AMPH
AMPH-GR
GR
PLACEBO

Food preference questionnaires were analysed pre and post test meal in order to assess subjective preference for macronutrients. The findings here reflected those seen with macronutrient intake. For analysis of forced choice protein vs carbohydrate, there was a significant main effect of time ($F_{1,21}$=53.19, $p<0.001$), with an increase in the selection of carbohydrate items post meal. There was also a significant main effect of amphetamine ($F_{1,21}$=5.76, $p<0.05$), which altered the ratio of carbohydrate to protein items in favour of carbohydrate items, and a significant time by amphetamine interaction ($F_{1,21}$=9.37, $p<0.01$). This was not true for GR68755 where there was no significant main effect of drug ($F_{1,21}$=0.73, $p>0.05$) or drug by time interaction ($F_{1,21}$=1.23, $p>0.05$). Thus, amphetamine suppressed selection of protein items pre meal. There was no significant interaction of drug condition ($F_{1,21}$=1.29, $p>0.05$), or of condition by time ($F_{1,10}$=0.8, $p>0.05$). (See Table 7.6 for mean and SD).

Similarly, for free choice of carbohydrate, protein and fat items there were also significant main effects of time, of amphetamine, with significant time by amphetamine interactions, no effects of GR68755, and no interactions of drug condition or condition by time. The effect of amphetamine on each of these measures was to decrease the selection of these items pre meal. The ANOVA findings are presented in Table 7.5, and means (±SD) are presented in Table 7.6).

7.4 DISCUSSION

The findings presented here confirm the hypophagic properties of d-amphetamine. Amphetamine suppressed total calorie intake at test meal, and this effect was not macronutrient specific, with significant reductions in carbohydrate, protein and fat consumption. These data are in keeping with previous research which describes amphetamine anorexia as acting to decrease the intake of all macronutrients (Silverstone, 1985; Blundell, 1985). The present study therefore confirms the effectiveness and validity of the test meal paradigm in measuring alterations in appetite. This alteration in food intake was accompanied by alterations in subjective ratings of hunger and appetite, which were similarly in keeping with the hypothesised effects of amphetamine.

As hypothesised, there was no independent effect of GR68755 on any of these measures. However, interestingly, there was also no effect of GR68755 on amphetamine induced alterations in appetite. There are several possible explanations for this lack of effect. Whilst it is true that animal models suggest an important role for 5-HT_3 blockade in inhibiting dopaminergic neurotransmission, and in inhibiting related behaviours (see Introduction for review), it is possible that amphetamine does not conform to these models. For example, whilst morphine- and nicotine-induced DA release were prevented by blockade of 5-HT_3 receptors (Jiang et al, 1990; Blandina et al, 1989; Wozniak et al, 1990), this was not true for amphetamine (Carboni et al, 1989a). This has also been found for behavioural responses to DA-releasing drugs; 5-HT_3 blockade can prevent the acquisition of conditioned behaviour responses to drugs such as morphine and nicotine (Carboni et al, 1989b; Higgins et al, 1992). However, this effect has not been demonstrated for amphetamine (Carboni et al, 1988, 1989b) or cocaine (Paris and Cunningham, 1991; Peltier

and Schenk, 1991).

It has been argued that this may be due to the fact that drugs like amphetamine do not act to increase firing of the DA neuron, but rather act directly on the dopaminergic synapse by displacing DA from synaptic terminals or by inhibiting synaptic reuptake (Carboni et al, 1989a/b). This is in contrast to drugs such as morphine and nicotine which may depend upon transmission at the excitatory 5-HT_3 receptors for their dopaminergic effects. These receptors would therefore theoretically be located presynaptically on the DA neuron. This theory, however, does not explain the findings demonstrating blockade of amphetamine-induced locomotor and exploratory behaviour (Costall et al, 1987; van der Hoek and Cooper, 1990b), and to date, the precise effects of 5-HT_3 antagonists on dopaminergic transmission remain unclear (Montgomery et al, 1993).

Amphetamine adminsitration is associated with increased extracellular 5-HT (see Introduction), and it has therefore been argued that amphetamine anorexia may be mediated in part via 5-HT release (Parada et al, 1988, 1992). Whilst 5-HT_3 receptors are concentrated particularly in the nucleus tractus solitarius within the brain stem (Pratt et al, 1990), they are present in lower densities within the limbic system (Kilpatrick et al, 1987). It is therefore possible that 5-HT_3 blockade could exert an independent effect on amphetamine anorexia via direct blockade of 5-HT at these sites. It would be of interest to examine this possibility using an animal model.

The pilot study in human volunteers (Silverstone et al, 1992) reported significant attenuation of hunger ratings across a morning using 15mg amphetamine and 4mg ondansetron. It is therefore surprising that the present study did not demonstrate this effect. However, it is

possible that the larger dose of amphetamine employed for the present study was responsible for the lack of effect. Furthermore, the relatively low dose of GR68755 may have failed significantly to block DA release within the mesolimbic system. The dosing regime adopted by Silverstone et al (1992) employed three consecutive doses of ondansetron spaced at 12 hour intervals with the last one occurring on the test morning. This design may have been more appropriate for the present study. A further consideration in the design of the present study is the length of observation; it is possible that the effects of GR68755 were not observed at the test meal because of the length of time subjects had been food-deprived. The rather high hunger ratings may have swamped any subtle alteration in hunger, thus GR68755 may well have exerted it's appetite-related effects at subsequent meals. An alternative methodology would have been to offer a standardised lunch followed by a test meal several hours later.

Cooper et al (1993) demonstrated an augmentation of amphetamine anorexia with ondansetron in rats, a finding which is not supported by the present study. Because of the large dose of amphetamine employed in the present study, it is possible that a floor effect was achieved (in some individuals calorie intake in the amphetamine condition was very modest), thus further calorie restriction would be unlikely. It would be of interest to examine the effects of 5-HT_3 blockade of amphetamine anorexia using a smaller dose.

Because of the potential role for DA in experiencing food as rewarding, it would be of interest to examine the effects of 5-HT_3 blockade on the hedonic and sensory responses to food and food intake. The present study did not aim to do this directly, but subjects were asked to rate meal satisfaction and desire to eat. Whilst these data do not support the theory that 5-HT_3 blockade modifies these aspects of reward, it would be of interest to

examine this area in more detail. For example, a design which required subjects to taste and rate preferences and responses to food stimuli in a controlled setting would be of interest.

In summary, whilst the present study does not support the role of 5-HT_3 blockade in amphetamine anorexia, this may be a result of possible limitations of the experimental design (ie dosing and period of observation); this approach would appear to be worth pursuing in the light of previous findings.

CHAPTER 8 GENERAL DISCUSSION

The experiments presented in this thesis were carried out in order to understand more fully the role of central 5-HT in the regulation of mood and appetite. Whilst much human literature supports an important role for 5-HT in mood, this comes mainly from psychiatric populations, with very little understanding of how 5-HT controls mood under normal circumstances, and furthermore, of the impact that behaviour (such as dieting) can have on these mechanisms. With regard to our understanding of appetite, there is a considerable body of animal literature which provides a valuable theoretical basis from which the role of 5-HT in human appetite regulation can be predicted. Human studies have concentrated on examining the anorectic effects of 5-HT drugs in a laboratory setting using healthy volunteers. There is an additional small number of studies attempting to examine dysregulated appetite in eating disordered populations. These studies, however, have been carried out on currently symptomatic clinical populations, and thus the data are of limited use in understanding the causal mechanisms which may be important in the development of disordered eating. One aim of the present thesis was therefore to examine the effects of manipulating brain 5-HT in healthy volunteers in order to understand further how perturbation of this system may result in alterations in mood and appetite including abnormal eating behaviour.

Chapter 3 demonstrated that a common behaviour, calorie-controlled dieting, has an important impact on levels of circulating tryptophan, a finding which has implications for brain 5-HT synthesis and function. Whilst tryptophan levels remain suppressed for the duration of the diet, they might normalise upon return to normal eating behaviour. It would be important to follow dieters over a period of time to establish that this was the case. The

finding of lowered plasma tryptophan has important implications for the large numbers of individuals (women in particular) who exercise moderate to high levels of restraint on a regular, day to day basis. As it is known that dieting compromises brain 5-HT function in women (Anderson et al, 1990b; Cowen et al, 1992), it would be of interest to pursue the impact of chronic restraint on plasma tryptophan and brain 5-HT function. The diet study, however, did not support the hypothesis that the changes in brain 5-HT function caused by short-term dieting would be associated with alterations in mood and appetite. However, one possible limitation of this study is that the measures of mood may well have been confounded by the positive effects of weight loss in the dieting group; because of the degree of commitment required for successful completion of the study, volunteers were highly motivated individuals who had a vested interest in weight loss, and furthermore, where applicable, experienced the delay aspect of the control condition as undesirable. It is also possible that short periods of dieting are not sufficient to elicit changes in mood. For example, it could be argued that the presence of an additional factor such as an adverse life event would be needed to translate the vulnerability into a clinical disorder. It would be of interest to carry out further work in populations who were not actively seeking to lose weight, and to examine the effects of longer term dieting. Appetite measures may also have been confounded by the constraints of the diet; dieters find it difficult to break the restriction necessary in order to adhere to their diet, and it therefore follows that responses to the test meal may well have been modified by the dieters' reluctance to break their dietary rules.

In response to the shortcomings of the above study, and in an attempt to examine further the effects of lowered tryptophan, the effects of tryptophan depletion were examined in Chapter 4 using an acute, laboratory-based manipulation. This design and methodology

provided the added advantage that subjects could act as their own controls. This manipulation, however, was not associated with alterations in mood or appetite. It is possible that the period of observation chosen for the study was not long enough to examine the hypothesised effects, but the data suggest that this explanation is unlikely. Alternatively, a short-term manipulation of this kind may not provoke adverse effects in that there may be a protective mechanism to control for short-term depletion. An alternative approach would have been to induce chronic tryptophan depletion via dietary means. Thus, subjects could be placed on a low tryptophan diet, with a crossover, or control comparison following the same diet plus tryptophan supplementation. Unfortunately this experiment was not possible because of the lack of availability of tryptophan during this time. A review of alternative control diets suggested that there was no acceptable alternative to tryptophan supplementation; tryptophan is a relatively rare amino acid, but is widely available in most foods, thus a tryptophan deficient diet would be very restrictive.

The lack of alteration in mood following tryptophan depletion conflicts with previous research (Young et al, 1985; Smith et al, 1987; Delgado et al, 1990). The measures adopted have been shown to be sensitive to subtle changes in mood, and are in keeping with those adopted by other research groups, suggesting that the effect was not present. However, because of the strict study entry requirements, the sample was not representative of the usual populations studied in that there was no history or family history of depression, and Beck Depression scores were very low. It is possible that the lower dose of amino acids employed in this experiment may have been insufficient to deplete brain tryptophan to the extent necessary to induce alterations in mood and appetite. However, recent studies using the larger dose have failed to demonstrate mood lowering effects in similarly carefully screened healthy volunteers (Young, pers comm). A population with no risk

factors to low mood may require an additional challenge to their mood state in order to induce a lowering of mood in the laboratory. One approach to this would be to introduce a form of 'mood manipulation' into the design of the experiment. This can be done, for example, with musical manipulation of mood or induction of negative affect by a process of reading lists of negative material. The impact of this manipulation could then be measured in response to tryptophan depletion compared with control. This raises the question of vulnerability to tryptophan depletion, an issue that was addressed by the experiment presented in Chapter 5.

The experiment carried out on women who had recovered from bulimia nervosa (Chapter 5) yielded several interesting findings. Most striking was the finding that this group had significantly lower baseline levels of total plasma tryptophan. This was an unexpected and novel finding, suggesting that this group may potentially have a pre-existing difference in at least one aspect of 5-HT metabolism; it is possible that this difference in tryptophan availability has implications for brain 5-HT synthesis and function. For example decreased precursor availability may result in decreased synthesis and transmission, unless adaptive changes within the 5-HT system occur. However, several limitations of this study need to be considered. Firstly, it must be noted that the experimental group was relatively small, and this population would have to be extended in order to confirm this finding. Secondly, it is possible that the eating behaviour of this group was responsible for these baseline differences. It would have been of interest to assess typical meal patterns and caloric intake for both groups in order that an estimation of how much the eating behaviour of the bulimic group differed from the expected norm. The study demonstrated that, on some measures, these subjects appeared to be showing patterns of restrained eating. It is possible, therefore, that these subjects were not fully 'recovered'. Whilst this group were free of clinical

symptoms, and rated themselves pre-study as having levels of restraint in keeping with the general population, it is possible that certain aspects of their eating behaviour were not fully normalised. Our method of assessment at initial screening could have been improved further by use of a structured interview schedule such as the Eating Disorders Examination (Cooper and Fairburn, 1987) in order to ascertain more fully their recovery status. However, it appears from the data collected in this experiment that differences exist at a sub-clinical level, and it may have been difficult to assess these with such a clinical instrument.

This highlights the need for further research in this area; very little data exists on the functioning of recovered eating disordered patients both in relation to the concept of 'what is recovery', and in relation to the process of recovery. It may be that for these individuals, 'recovery' is a state of 'control' over eating which is determined by processes of cognitive restraint which are different to those of highly restrained individuals in the general population. A comparison of high restraint versus recovered bulimic patients would further our understanding of the potential role of restrained eating in influencing levels of plasma tryptophan, and furthermore, in the patterns of eating behaviour exhibited by these groups. The addition of a normal control group in such a study would clarify the interpretation of such findings. For example, little is known about the effects of chronic restraint on plasma tryptophan and brain 5-HT function. If the decreased baseline difference in plasma tryptophan seen in recovered bulimic patients could be successfully replicated, then it would be important to assess whether this lowered precursor availability had any functional consequences for brain 5-HT neurotransmission. This issue could be pursued, for example, using a neuroendocrine challenge paradigm. Previous research suggests that, if available, the most appropriate challenge agent would be L-tryptophan (Anderson et al, 1990b; Cowen

et al, 1992).

Further analysis of the differences in eating behaviour between recovered bulimic patients and normal controls would be important in furthering our understanding of how patients achieve and maintain a state of relative recovery. At present it is unclear whether these individuals return to normal eating behaviour, or, as the data from this study suggest, learn to cope effectively with tendencies towards disordered eating by, for example, using forms of cognitive and behavioural restraint to control their intake; it is possible that individuals adhere strictly to dietary rules such as number of meals, amounts and types of food that may be eaten, and disregard internal hunger cues. A study which addressed the issues of recovery and maintenance of recovery would have implications for the development of psychological treatment interventions with this population.

Chapter 5, however, did not report a vulnerability of recovered bulimic patients to tryptophan depletion for either changes in mood or appetite. This does not support a role for dietary-induced changes in 5-HT function in appetite dysregulation. However, the criticisms cited for the previous study are also applicable here; it is possible that an acute manipulation of this kind is not sufficient to disrupt the 5-HT mechanisms involved in appetite regulation. Again, a more chronic intervention to lower plasma tryptophan would be of interest in this population.

The above studies did not support the hypothesis that tryptophan depletion would alter mood and appetite in healthy or vulnerable individuals, suggesting that there may be a protective mechanism against short-term depletion. The remaining chapters therefore sought to explore the role of specific 5-HT receptor subtypes in appetite regulation. Chapter 6

demonstrated that oral administration of mCPP was associated with increases in prolactin and temperature, indicating that this drug was centrally active at 5-HT receptor sites. There was however, no alteration in appetite in this study. This is the first time that the anorectic properties of mCPP have been examined in the human, and it is therefore necessary to interpret this negative finding cautiously. There appear to be two principal explanations for this lack of effect; firstly, as discussed previously (see Chapter 6) mCPP may not act in the same way within the human CNS as described by the animal models, thus failing to elicit a hypophagic response. Alternatively, it is possible that the doses employed for this investigation were too conservative to produce the response. This explanation is supported by a subsequent study (Walsh et al, pers comm) in which higher doses of mCPP (0.4mg/kg) were associated with decreased food intake at the test meal. Further research is therefore needed to establish whether mCPP produces a true anorectic effect, or whether this is secondary to mCPP-induced symptoms such as nausea and anxiety. From a clinical perspective, it would also be of interest to examine the effects of mCPP on macronutrient selection; for example, d-fenfluramine has been of clinical importance because of its claimed potential to reduce carbohydrate snacking, especially in obese populations (Wurtman et al, 1985), and historically, amphetamine has been employed in weight reduction programmes because of its ability to suppress appetite globally (Silverstone and Stunkard, 1968). From a theoretical perspective, it still remains unclear whether drugs affecting appetite can act specifically or principally upon a single macronutrient.

The final experimental chapter (Chapter 7) examined the relationship between brain 5-HT function and NA/DA-mediated appetite suppression. This experiment replicated the appetite suppressant effects of amphetamine, but failed to show an effect of 5-HT_3 blockade upon this. There appear to be two likely explanations for this lack of effect. Firstly, a pilot

study in humans using ondansetron reported attenuation of amphetamine anorexia with a lower dose of amphetamine (Silverstone et al, 1992). It is therefore possible that the appetite suppressant effects of our 20mg dose of amphetamine could not be blocked by 2mg of GR68755. Alternatively, it remains unclear from the animal literature whether the effects of amphetamine on dopamine release can be attenuated by 5-HT_3 blockade in the same way as drugs known to enhance dopaminergic neurotransmission (Montgomery et al, 1993). Whilst the precise role of dopamine in amphetamine anorexia remains unclear, the current literature suggests that this effect may be at least partly dopaminergic (see Chapter 7). However, animal findings suggest that it is easier to attenuate the behavioural effects of dopaminergic drugs such as nicotine and morphine with 5-HT_3 blockade (Montgomery et al, 1993). It would therefore be of theoretical interest to examine the effects of 5-HT_3 blockade on these drugs in humans.

The Measurement of Appetite

The present thesis adopted a free choice, buffet style test meal paradigm for the measurement of appetite. This was used in conjunction with VAS ratings of appetite, and food preference questionnaires. The test meal appears to be an effective and sensitive measure of changes in appetite. For example, it was found that the total number of calories consumed after a 50g amino acid loading was significantly lower than placebo at +4.5 hours (see Chapter 5). Furthermore, there was a significant reduction of calorie consumption by amphetamine at test meal (Chapter 7). Employment of this particular methodology allowed analysis of both total calorie and macronutrient intake. However, a possible shortcoming of this method of food intake analysis is that offering such a variety of foods may confound the experimental effect. Thus experimentally induced, subtle alterations in appetite may be counteracted by subjects selecting widely from the palatable foods on offer. It has been

shown, for example, that the sensory properties of foods will directly affect food intake in a laboratory setting (Rolls et al, 1982).

Alternatively, researchers have adopted a more simplistic approach to the measurement of food intake; meals have consisted of a more limited choice of food types, such as sandwiches or crackers (see Silverstone and Fincham, 1978 for review). Here it is argued that the relatively mundane nature of the foods prevents food intake for reasons other than satiation of hunger. Whilst this methodology may be more sensitive in the measurement of total calorie intake, this does not allow for a consideration of macronutrient intake, an important theoretical consideration of the present thesis. Moreover, this methodology has been criticised for not being naturalistic and therefore not applicable to real eating behaviour outside of the laboratory (Silverstone and Fincham, 1978).

A further limitation of the test meal methodology is that it does not allow measurement of the time course of the experimental effect. That is, the manipulation may produce an effect only at certain stages of the meal or period of eating, with no overall change in a particular macronutrient or total calories consumed. A methodology which overcomes this limitation involves use of an automated food dispenser from which subjects are free to select items at any time during the experimental session (Goodall and Silverstone, 1988; Goodall et al, 1991). This methodology enables accurate recording of food intake at specified time periods in addition to overall intake across a session, thus overcoming the limitations of a single meal presentation.

Subjective ratings of hunger using VAS scales have proved to be highly sensitive to change, and to correlate well with actual intake (Silverstone and Fincham, 1978). The studies

presented in the present thesis confirmed that VAS scales are an effective way to measure hunger, in addition to other aspects of appetite such as 'desire to eat' and 'feelings of fullness'. This method proved highly sensitive to time effects, food consumption, and to group differences. Furthermore, this method of rating was successfully applied to the measurement of abnormal eating patterns, and yielded interesting group differences (Chapter 5).

The free and forced choice questionnaires (Hill, 1987) provided an additional measurement of macronutrient selection. These measures proved equally sensitive to the effects of time, food selection, and to group differences. They do, however, have some limitations; these measures cannot be used with vegetarian subjects, thus causing a bias in the population sample studied. With the increasing numbers of people with restrictive diets, it is questionable whether it is appropriate to have this as an exclusion criterion. These measures may be appropriate in experimental situations where 'actual' macronutrient intake cannot be measured, but they did not appear to offer any additional information to the measures adopted in the present thesis. Interpretation of the forced choice questionnaire (carbohydrate vs protein) is further restricted because of the lack of control for the fat content of the food items included on this measure.

A review of the above area raises the question of whether it is indeed possible to observe and measure alterations in macronutrient selection, and whether these short-term adaptations in macronutrient selection do, in fact, exist. Any CNS mechanism postulated to influence macronutrient selection could only be observable if the organism can respond rapidly to a manipulation of that mechanism. That is, feedback would have to be very quick in order that the necessary adjustments could be made. It therefore follows, for example, that the

effects of a pharmacological manipulation postulated to suppress carbohydrate intake could only be observed if the individual was in a position to make food choices which were informed by the nutritional content of the foods on offer. Animal experiments have shown that before such choices can be made, the animal has to learn which sensory characteristics of a particular diet predict the effects of, for example high or low carbohydrate or protein content. Only then can nutrient selection occur (Gibson and Booth, 1988). This line of argument would suggest that it is unlikely that macronutrient intake is regulated on a meal to meal basis, but that these mechanisms act over a longer period of time to maintain a status of optimum nutrition. Furthermore, it has been argued that beliefs and intentions about nutrition and about the foods offered interfere with the physiological mechanisms controlling nutrient selection, thus cognitive and physiological influences may be operating in opposite directions in the experimental setting (Booth et al, 1989). It seems likely that, in a test meal, an individual may continue to select a 'typical' meal from the buffet in response to external cues such as 'time of day', and their beliefs about their nutritional needs.

Whilst it is difficult to envisage a strategy for disentangling physiological and cognitive influences on food consumption, various methodologies exist which enable a more naturalistic assessment of the foods consumed by an individual. This can be done by asking an individual to keep precise dietary records of all the foods they eat over a given time (Garrow et al, 1993). In addition, if subjects are specifically trained, the use of weighing scales further enhances the quality of data collected. However, it has been argued that there are severe limitations with both of these methods, and that they are too inaccurate for detailed analysis of intake (Bingham, 1988). It would therefore appear that for the purposes of the investigations described in the present thesis, a test meal methodology was

the most appropriate way to examine the hypotheses in question. It may, however, have been of interest to follow subjects over a longer period of time in order to assess the impact of the acute manipulations on food intake for the whole of the day.

5-HT, Mood and Appetite

The present thesis does not support a straightforward association between manipulations of brain 5-HT and subsequent alterations in mood or appetite. However, one aim of the present thesis was to further an understanding of the underlying mechanisms regulating mood and appetite by applying a 5-HT model to this process of regulation (see Fig 6.1, page 100). Two approaches have been adopted to this end: first, the impact of behaviour (in this case, dieting) upon this model was explored. Second, the effects of manipulations of tryptophan and 5-HT on behaviour and psychological variables (ie mood and appetite) were examined in relation to this model. For example, it was demonstrated that dieting was associated with alterations in plasma tryptophan. It remains to be established what the effects of a more chronic lowering of plasma tryptophan may be in terms of alterations in mood and appetite. This and previous research (Anderson et al, 1989; Goodwin et al, 1990; Cowen et al, 1992) suggest that dieting behaviour can have an important impact on central brain 5-HT function, especially in women. These findings have potential implications for our understanding of the development of clinical eating disorders; for example, it remains to be established whether CNS disturbances in neurotransmitter function associated with anorexia and bulimia nervosa precede the disorder, or are a consequence of chaotic eating. The present data suggest that eating behaviour could have a significant impact on central neurotransmitter function. This suggestion is further supported by the finding that women who have recovered from bulimia nervosa have lower baseline levels of plasma tryptophan

than controls.

Whilst much animal and human literature supports a strong relationship between enhanced 5-HT transmission and decreased appetite and food intake, the relationship between decreased 5-HT availability and appetite is much less clear. The present thesis supports the notion that acute reductions in precursor availability are not associated with alterations in appetite. Existing evidence suggests that the manipulation carried out for these studies is associated with decreased 5-HT availability and synthesis, and it can therefore be tentatively concluded that the present findings suggest decreased 5-HT function may not be associated with an hypothesised increase in appetite. Whilst there are limitations to the design adopted (see specific chapter discussions and above), it seems likely that in normal functioning there may be a 'defence' mechanism which protects the brain against short-term depletions in precursor availability, and furthermore, that this mechanism does not involve short-term adjustment of nutrient intake in order to restore the balance. Chronic interventions known to decrease brain 5-HT function clearly do appear to lead to the hypothesised increase in appetite. For example 5-HT receptor blockers such as cyproheptadine and clozapine are associated clinically with unwanted weight gain (Silverstone and Schuyler, 1975).

The role of 5-HT receptor subtypes in the control of appetite in the human remains to be elucidated. Whilst the present thesis does not directly support a role for the 5-HT_{2C} receptor, subsequent work suggests that this receptor subtype may indeed play a role in appetite regulation. Confirmation of this finding would suggest that this receptor may be an important focus for developing pharmacological appetite suppressants. A fuller exploration of the potential role of the 5-HT_{2C} receptor in this field, however, awaits the

development of more selective compounds. A second area for future research involves furthering our understanding of the relationship between 5-HT and other neurotransmitters associated with the regulation of appetite. The present thesis did not demonstrate an association between 5-HT_3 blockade and amphetamine anorexia (DA- and NA- mediated), but it would be important to explore further the potential of 5-HT_3 blockade upon DA- and NA-induced hypophagia. Because of the apparent resistance of amphetamine-induced responses to 5-HT_3 blockade, this may best be carried out with other dopaminergic and noradrenergic drugs.

In conclusion, the present thesis describes a series of experiments in humans which support the suggestion that 5-HT precursor availability can be lowered by behaviour and pharmacological manipulation, but that this is not associated with alterations in mood or appetite in the human. Findings from these studies suggest that disturbance in normal eating patterns may be associated with alterations in tryptophan availability, thus suggesting a hypothetical role for this in clinical eating disorders. This thesis further suggests that current knowledge is not sufficient to describe a role for specific 5-HT receptor subtypes in the control of appetite in the human, but that further research would clarify this important area.

APPENDICES 2-6 APPENDICES FOR CHAPTERS 2 TO 6

(Appendix numbers correspond to chapter numbers, hence these begin with Appendix 2)

APPENDIX 2.1

FOOD PREFERENCES

NAME: _____

CODE: _____

Please rate the following foods according to your preferences, using the scale below:

1	2	3	4	5	6	7	8	9	10
Dislike Extremely			Neutral				Like		

Bread:

_____White

_____Soft Grain (Mighty White)

_____Wholemeal

Sandwich Fillings and cold meats:

_____Cheddar Cheese

_____Cottage Cheese

_____Peanut Butter

_____Ham

_____Tuna Fish

_____Chicken Roll

_____Marmite

_____Strawberry Jam

Chocolate:

_____Milk Chocolate (Cadbury's)

_____Plain Chocolate (Bournville)

_____White Chocolate (Milky Bar)

Biscuits:

_____Rich Tea

_____Morning Coffee

_____Digestive

Desserts:

_____Strawberry Yoghurt

_____Raspberry Yoghurt

_____Black Cherry Yoghurt

_____Fruit salad (tinned)

_____Rice pudding

Crisps:

_____Ready Salted

_____Smoky Bacon

_____Cheese and Onion

Fruit:

_____Apple

_____Orange

_____Banana

Appendix 2.2: COMPOSITION OF TEST MEAL			
	CHOICE RANGE*	QUANTITY	APPROX WEIGHT
Bread	wholemeal, white, softgrain	8 slices of one type	270g
Margarine		small tub	250g
Sandwich Fillings	ham, tuna, chicken roll, cheddar cheese, cottage cheese, peanut butter, marmite, jam	4 choices (minimum of 2 to be protein)	eg: 230g tuna; 250g cottage cheese; 100g ham; 340g peanut butter
Crisps	3 flavours	2 pkts of one type	60g
Pudding	yoghurt, rice pudding, fruit salad	1 type	eg: 270g yoghurt
Fruit	apple, banana, orange	1 item each of 2 types	eg: 120g apple, 100g banana
Biscuits	digestive, rich tea, morning coffee	1 type	120g
Chocolate	milk, plain, white	12 squares of one type	75g
Tomatoes		2	140g

*choice determined by food preference ratings completed by volunteer prior to study

Appendix 2.3: COMPOSITION OF TEST MEAL FOR MALE VOLUNTEERS			
	CHOICE RANGE*	QUANTITY	APPROX WEIGHT
Bread	wholemeal, white, softgrain	10 slices of one type	340g
Margarine		small tub	250g
Sandwich Fillings	ham, tuna, chicken roll, cheddar cheese, cottage cheese, peanut butter, marmite, jam	4 choices (minimum of 2 to be protein)	eg: 230g tuna; 250g cottage cheese; 120g ham; 340g peanut butter
Crisps	3 flavours	3 pkts of one type	90g
Pudding	yoghurt, rice pudding, fruit salad	1 type	eg: 400g yoghurt
Fruit	apple, banana, orange	2 items of 2 types	eg: 180g apple, 150g banana
Biscuits	digestive, rich tea, morning coffee	1 type	150g
Chocolate	milk, plain, white	16 squares of one type	100g
Tomatoes		2	140g

*choice determined by food preference ratings completed by volunteer prior to study

Appendix 2.2 (cont): Descriptive summary and nutritional breakdown of food items included in test meals.

CATEGORY	FOOD ITEM	APPROX ENERGY (KJ/100g)	CHO E%	FAT E%	PROTEIN E%
CARBOHYDRATE	White Bread	926	80.9	5.2	14.0
	Soft Grain Bread	978	81.1	5.7	13.2
	Wholemeal Bread	915	72.8	10.1	17.1
	Strawberry Jam	1114	99.1	0.0	0.9
	Rice Pudding	374	59.8	24.7	15.4
	Fruit Salad	122	94.4	0.0	5.6
	Biscuit - Rich Tea	1925	62.2	31.9	5.9
	Biscuit - Morning Coffee	1891	62.3	31.9	5.8
	Biscuit - Digestive	1978	55.5	39.1	5.4
	Yoghurt - Low Fat	369	69.0	7.0	24.0
	Tomato	73	68.3	15.3	16.4
	Apple	199	94.7	1.9	3.4
	Orange	158	85.9	2.3	11.8
	Banana	403	92.2	2.8	5.1
FAT	Cheddar Cheese	1708	0.1	74.5	25.4
	Peanut Butter	2581	8.1	77.0	14.9
	Milk Chocolate	2176	43.2	50.8	6.0
	Plain Chocolate	2197	47.2	49.2	3.6
	White Chocolate	2212	42.2	51.7	6.1
	Crisps	2106	30.5	64.3	5.2
	Margarine	2984	0.7	99.2	0.1
PROTEIN	Cottage Cheese	413	8.1	35.0	56.9
	Ham	385	0.4	19.2	80.4
	Tuna Fish	488	0.3	8.3	91.3
	Chicken Roll	767	0.0	35.2	64.8
	Marmite	730	3.9	3.5	92.5

APPENDIX 2.4 RECORD SHEET

Subject Name: _____ Date: _____

Code: _____ Time Taken: _____

FOOD	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT CONSUMED
Bread ----------			
Margarine			
Filling 1:- ----------			
Filling 2:- ----------			
Protein 1:- ----------			
Protein 2:- ----------			
Pudding ----------			
Crisps			
Fruit 1:- ----------			
Fruit 2:- ----------			
Biscuit:- ----------			
Chocolate:- ----------			
Tomato			
TOTALS:-			

APPENDIX 2.5 PRINTOUT OF CALCULATION OF TEST MEAL FROM 'DIRECT'

EXPERIMENT smith2 — 29 APR 1993

Subjects Name or Code ....
Subjects PIN Code 2
Total Experiment Duration - 30 Minutes
Counting Period Length - 5 Minut
Experiment Details
1of1

WEIGHT TABLE

Tray	Description of Food	Swt/Sa	Weight	g/100g Prot	g/100g Fat	g/100g Carb
1	bread Allinsons wm	sav	69.3	10.1	2.6	38.7
2	marg	sav	3.6	0.2	80	1.3
3	ham sainsburys	sav	65.0	18.2	2.0	0.1
4	yoghurt sa blk ch	sw	215.1	5.2	0.7	15.9
5	crisps	sav	33.1	6.5	36.6	40.1
6	apple	sw	132.6	0.4	0.1	11.8
7	banana	sw	46.5	1.2	0.3	23.2
8	cottage cheese	sav	150	13.7	4.0	1.60
9	cheddar cheese	sav	24.0	25.5	34.0	0.1
10	tomato	sw	140.4	0.7	0.3	3.1

ENERGY TABLE

Tray	Description of Food	Total KJ	Protein KJ (%)	Fat KJ (%)	Carbo's KJ (
1	bread Allinsons wm	615	119.0 (19.4%)	66.7 (10.8%)	429.1 (69.8
2	marg	107	0.1 (0.1%)	106.6 (99.2%)	0.7 (0.7%
3	ham sainsburys	250	201.1 (80.4%)	48.1 (19.2%)	1.0 (0.4%
4	yoghurt sa blk ch	793	190.1 (24.0%)	55.7 (7.0%)	547.2 (69.0
5	crisps	697	36.6 (5.2%)	448.2 (64.3%)	212.4 (30.5
6	apple	264	9.0 (3.4%)	4.9 (1.9%)	250.3 (94.7
7	banana	187	9.5 (5.1%)	5.2 (2.8%)	172.6 (92.2
8	cottage cheese	610	349.4 (57.3%)	222.0 (36.4%)	38.4 (6.3%
9	cheddar cheese	406	104.0 (25.6%)	301.9 (74.3%)	0.4 (0.1%
10	tomato	102	16.7 (16.4%)	15.6 (15.3%)	69.6 (68.3

EXPERIMENTAL RESULTS

Data entered manually, 29 APR 1993

Final Results

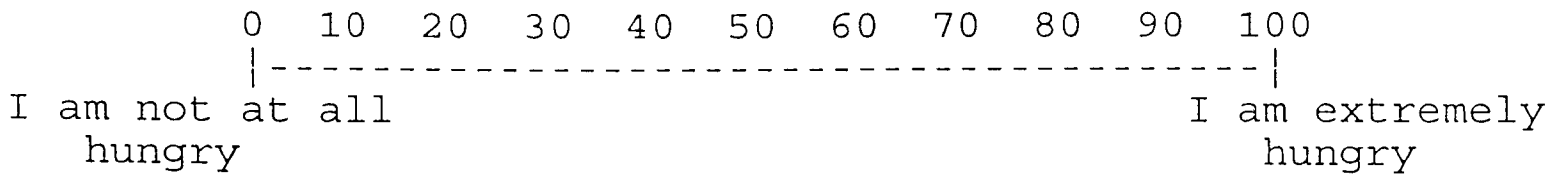
Tray	Description of Food	Category	Count	Weight	E(Prot)	E(Fat)	E(Carbo)
1	bread Allinsons wm	Savoury	1.00	69	119.0	66.7	429.1
2	marg	Savoury	1.00	4	0.1	106.6	0.7
3	ham sainsburys	Savoury	1.00	65	201.1	48.1	1.0
4	yoghurt sa blk ch	Sweet	1.00	215	190.1	55.7	547.2
5	crisps	Savoury	1.00	33	36.6	448.2	212.4
6	apple	Sweet	1.00	133	9.0	4.9	250.3
7	banana	Sweet	1.00	47	9.5	5.2	172.6
8	cottage cheese	Savoury	1.00	150	349.4	222.0	38.4
9	cheddar cheese	Savoury	1.00	24	104.0	301.9	0.4
10	tomato	Sweet	1.00	140	16.7	15.6	69.6

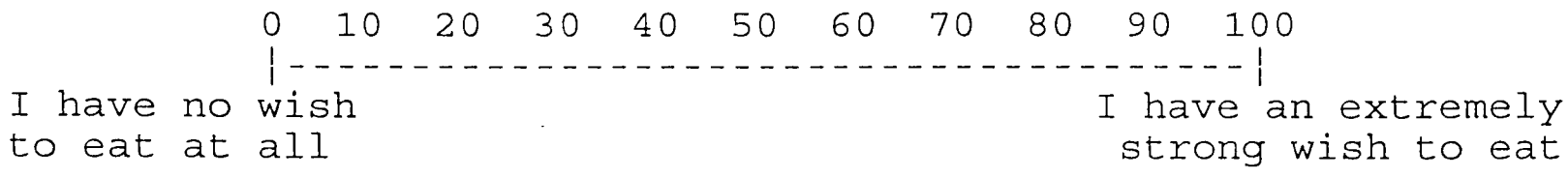
Energy from	Sweet KJ	(%)	Savoury KJ	(%)	Total KJ	(%)
Protein	225.36	5.59%	810.19	20.09%	1035.55	25.68%
Fat	81.36	2.02%	1193.49	29.60%	1274.85	31.62%
Carbohydrate	1039.81	25.79%	682.05	16.91%	1721.86	42.70%
Total	1346.53	33.39%	2685.72	66.61%	4032.25	100.00%

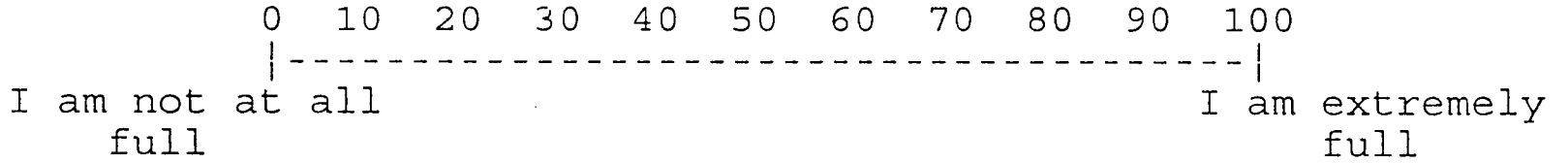
APPENDIX 2.6

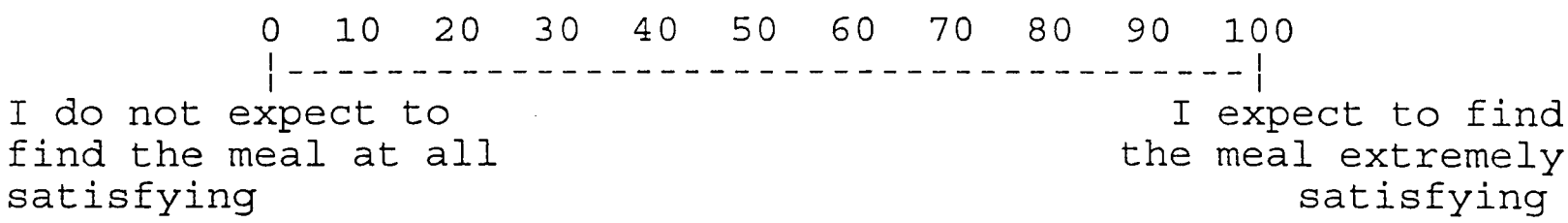
VISUAL ANALOGUE RATING SCALES FOR APPETITE ITEMS - PRE-MEAL RATINGS

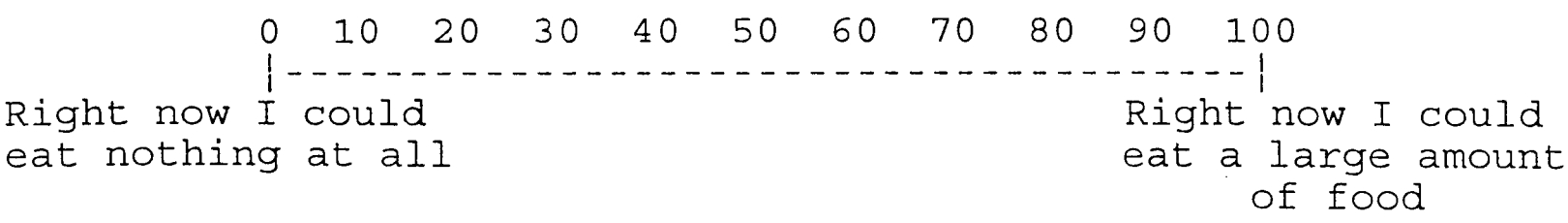
PLEASE EITHER PLACE A MARK ON THE LINE OR CIRCLE THE NUMBER WHICH BEST INDICATES HOW YOU FEEL <u>RIGHT NOW</u>











APPENDIX 2.6 continued

VISUAL ANALOGUE RATING SCALES FOR APPETITE ITEMS - POST-MEAL RATINGS

PLEASE EITHER PLACE A MARK ON THE LINE OR CIRCLE THE NUMBER WHICH BEST INDICATES HOW YOU FEEL RIGHT NOW

0 10 20 30 40 50 60 70 80 90 100

I am not at all hungry — I am extremely hungry

0 10 20 30 40 50 60 70 80 90 100

I have no wish to eat at all — I have an extremely strong wish to eat

0 10 20 30 40 50 60 70 80 90 100

I am not at all full — I am extremely full

0 10 20 30 40 50 60 70 80 90 100

I do not expect to find the meal at all satisfying — I expect to find the meal extremely satisfying

0 10 20 30 40 50 60 70 80 90 100

Right now I could eat nothing at all — Right now I could eat a large amount of food

APPENDIX 2.7 FREE CHOICE FOOD PREFERENCE QUESTIONNAIRE

SUBJECT .....

Examine each individual item in turn to make your assessment. If you would like to eat it at this moment place a cross on the line next to it. If not, go on to the next. Consider each item independently from the others. Do not spend a long time on any item.

---- A roast chicken breast
---- A current bun
---- A large Cadburys Flake
---- A medium-sized peach
---- A baked potato with a small knob of butter
---- A small dish of fried mushrooms
---- A medium size grilled cod fillet
---- Two average size tomatoes
---- A grilled lean lamb cutlet
---- A small slice of cheesecake
---- A small green salad
---- A crusty white or brown bread roll
---- Two slices of corned beef
---- Four ginger biscuits
---- A medium size sausage roll
---- A dish of fresh stawberries
---- Half a cup of tinned salmon
---- Two pickled onions
---- A small slice of jam-filled sponge cake
---- A grilled lean piece of gammon
---- Two lemon pancakes
---- A medium size dish of baked beans
---- A carton of natural yoghurt
---- One and a half packets of crisps (any flavour)
---- A dish of shelled prawns
---- A dish of tinned fruit salad
---- A 2oz wedge of cheddar cheese
---- A grilled $^{1}/_{4}$lb rump steak
---- Two sticks of celery
---- A cream filled chocolate eclair
---- A medium size bowl of fried rice
---- A small slice of honeydew melon

Appendix 2.8: Descriptive summary and nutritional breakdown of food items included in 32 item free choice food preference checklist.

CATEGORY	FOOD ITEM	APPROX ENERGY (KCAL)	CHO E%	FAT E%	PROTEIN E%
CARBOHYDRATE	A crusty white or brown bread roll	200	74.0	10.0	16.0
	A medium size dish of baked beans	180	60.8	7.1	32.1
	A baked potato with a small knob of butter	205	62.7	30.4	6.9
	A medium size bowl of fried rice	190	58.7	28.8	12.5
	4 ginger biscuits	175	65.1	30.0	4.9
	A small slice of jam filled sponge cake	200	79.7	14.7	5.6
	A dish of tinned fruit salad	180	98.7	0.0	1.3
	A currant bun	220	67.7	22.5	9.8
	Mean	194	71.0	17.9	11.1
FAT	One and a half packets of crisps (any flavour)	200	34.7	60.6	4.7
	A small dish of fried mushrooms	180	0.0	95.7	4.3
	A medium size sausage roll	210	25.9	68.1	6.0
	A two ounce wedge of cheddar cheese	225	0.0	74.4	25.6
	A small slice of cheesecake	210	21.4	74.6	4.0
	A cream-filled chocolate eclair	205	38.1	57.5	4.4
	A large Cadbury's flake	180	42.0	51.6	6.4
	2 lemon pancakes	210	32.9	57.3	9.8
	Mean	203	24.3	67.5	8.2
PROTEIN	A grilled lean lamb cutlet	210	0.0	49.8	50.2
	A grilled lean piece of gammon	200	0.0	27.0	73.0
	Half a cup of tinned salmon	185	0.0	47.6	52.4
	A medium size grilled cod fillet	180	0.0	12.3	87.7
	A grilled 1/4 lb rump steak	205	0.0	32.0	68.0
	A dish of shelled prawns	180	0.0	15.3	84.7
	A roast chicken breast	200	0.0	25.4	74.6
	2 slices of corned beef	180	0.0	50.2	49.8
	Mean	193	0.0	32.4	67.6
LOW ENERGY	A small green salad	6			
	2 average size tomatoes	14			
	2 sticks of celery	10			
	2 pickled onions	8			
	A medium size peach	36			
	A small slice of honeydew melon	25			
	A dish of fresh strawberries	20			
	A carton of natural yoghurt	80			
	Mean	25			

APPENDIX 2.9 FORCED CHOICE FOOD PREFERENCE QUESTIONNAIRE

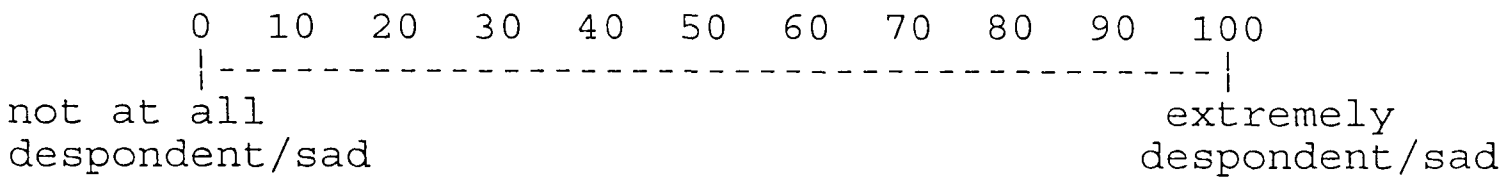
SUBJECT .....

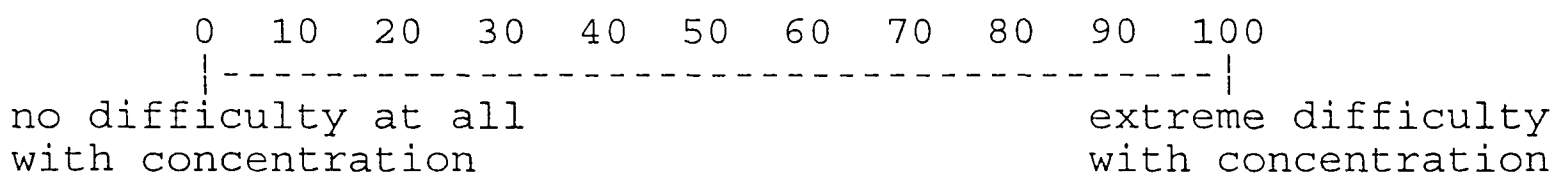
Please examine each of the following pairs of food items one at a time and place a cross on the line next to the one which you would prefer to eat at this moment. You much indicate your preference for one food item out of each pair.

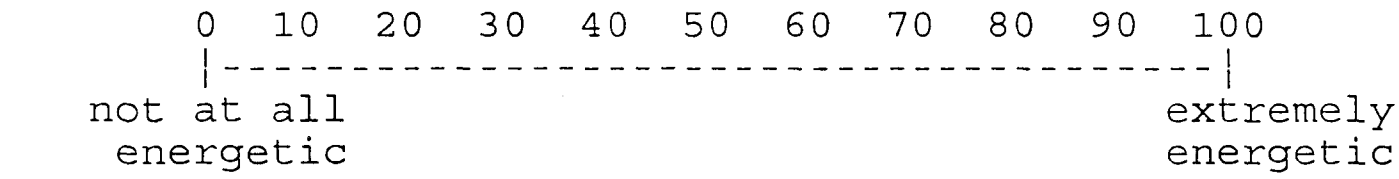
A roast chicken breast	---- or ----	A baked potate with butter
1/4lb grilled rump steak	---- or ----	A large Cadburys Flake
A doughnut	---- or ----	A medium size grilled cod fillet
2oz of cheddar cheese	---- or ----	A dairy king cone ice cream
A dish of tinned fruit salad	---- or - ---	Two poached eggs
A baked potato with butter	---- or ----	1/4lb grilled rump steak
2oz of cheddar cheese	---- or ----	A doughnut
A dairy king cone ice cream	---- or - ---	A roast chicken breast
A baked potato with butter	---- or - ---	A medium size grilled cod fillet
Two poached eggs	---- or ----	A large cadburys Flake
1/4lb grilled rump steak	---- or ----	A doughnut
A dish of tinned fruit salad	---- or ----	2oz of cheddar cheese
A roast chicken breast	---- or ----	A large Cadburys Flake
A baked potato with butter	---- or ----	Two poached eggs
A medium size grilled cod fillet	---- or ----	A dairy king cone ice cream
1/4lb grilled rump steak	---- or ----	A dish of tinned fruit salad
A large Cadburys Flake	---- or ----	A medium size grilled cod fillet
A roast chicked breast	---- or ----	A dish of tinned fruit salad
A doughnut	---- or ----	Two poached eggs
2oz of cheddar cheese	---- or ----	A baked potato with butter
A dairy king cone ice cream	---- or ----	1/4lb grilled rump steak
A medium size grilled cod filled	---- or ----	A dish of tinned fruit salad
Two poached eggs	---- or ----	A dairy king cone ice cream
A doughnut	---- or ----	A roast chicken breast
A large Cadburys Flake	---- or ----	2oz of cheddar cheese

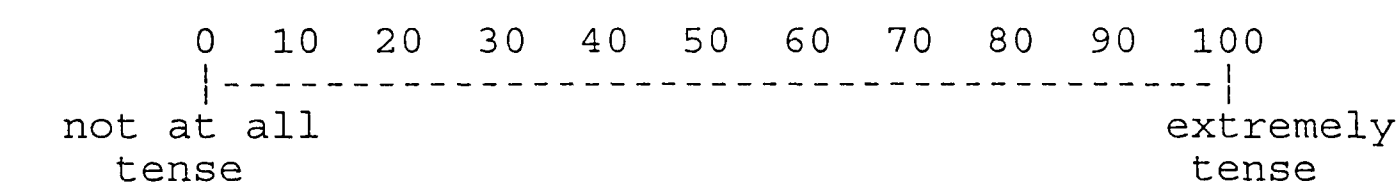
APPENDIX 2.10 VISUAL ANALOGUE SCALES OF MOOD

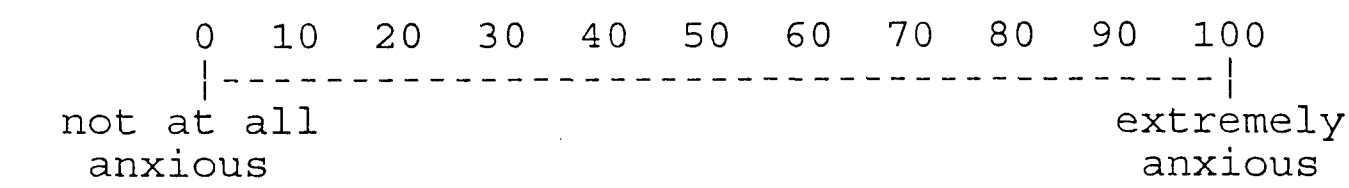
PLEASE EITHER PLACE A MARK ON THE LINE OR
CIRCLE THE NUMBER WHICH BEST INDICATES HOW
YOU FEEL <u>RIGHT NOW</u>

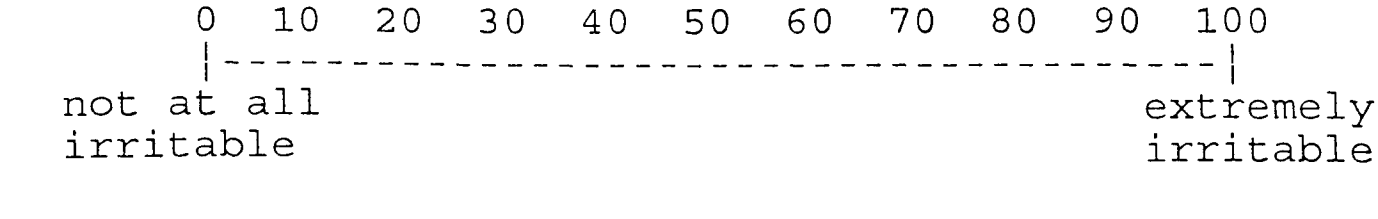












APPENDIX 3.1 1000 KCAL DIET - INSTRUCTIONS AND EATING PLAN

1000 KCAL DIET FOR DIET STUDY

TOTAL DAILY INTAKE:

1 pint skimmed milk
1/2 oz (2 level teaspoons) of low fat spread
3 meat exchanges
4 bread exchanges
2 portions of fruit (without sugar)
plenty of freely allowed foods.

****

DAILY EATING PLAN:

Milk allowance: 1 pint skimmed milk
Fat allowance: 1/2 oz (2 level teaspoons low fat spread)

Breakfast:

Breakfast cereal (or 1 bread exchange)
1 slice wholemeal bread (or 1 bread exchange)
Tea or coffee
Milk and fat from allowance

Mid-morning:

Low calorie drink or tea or coffee with milk from allowance

Lunch:

2 oz lean meat (or 1 meat exchange)
Large serving salad or freely allowed vegetables
1 slice wholemeal bread (or 1 bread exchange)
Fruit - fresh or stewed without sugar
tea or coffee

Tea:

Low calorie drink or tea or coffee with milk from allowance

Evening Meal:

4 oz lean meat (or 2 meat exchanges)
Large serving salad or freely allowed vegetables
1 small potato (or 1 bread exchange)
Fruit - fresh or stewed without sugar
Tea or coffee

Bed-Time:

Tea or coffee with rest of milk allowance

COOKING METHODS:

Meats:

Grill, roast or casserole any lean meat (add lots of vegetables to casseroles)

Fish:

Grill (using a small knob of butter or margarine from allowance), bake in the oven with skimmed milk or natural yoghurt, try adding mushrooms and tomatoes too

Eggs:

Boiled, poached or scrambled, using minimum amount of fat

MEAT EXCHANGES

1 Meat Exchange:
2 oz lean meat or chicken
2 oz oily fish (drain off excess oil)
4 oz white fish
1 oz (matchbox size) hard cheese
3 oz cottage cheese
1 egg
5 oz carton natural yoghurt

BREAD EXCHANGES

1 Bread Exchange:
1 small slice wholemeal bread
2 wholemeal crispbreads (eg Ryvita)
1 serving breakfast cereal: (1 Weetabix, 1 oz Puffed Wheat, 1 Shredded Wheat, 1 oz Bran Flakes, 1 oz All Bran, 4 oz porridge)
1 small potato
1 1/2 oz (3 tablespoons) cooked brown rice
2 1/2 oz (3 tablespoons) baked beans

FREELY AVAILABLE FOODS

The following foods are very low in calories and you can fill up on these:-

Fruit

Grapefruit, melon, rhubarb, gooseberries, loganberries, blackberries, lemon, redcurrants and blackcurrants.

Vegetables

Asparagus, aubergine, french beans,runner beans, bean sprouts, bamboo shoots, broccoli, brussel sprouts, courgettes, cabbage, carrots, cauliflower, celeriac, celery, chicory, chives, chinese cabbage, cucumber, leeks, lettuce, marrow, mushrooms, mustard and cress, onions, peas, peppers, pumpkin, radishes, spinach, spring greens, swede, tomatoes, turnip, watercress.

Condiments

Mustard, pepper, worcester sauce, curry powder, vinegar, clear pickles, herbs and spices, tomato juice, clear soup, Bovril, Marmite, Oxo, soy sauce

Tablet and liquid saccharine sweetener (avoid powdered sweeteners as they often contain sugar)

Drinks

Sugar-free drinks, including Boots diabetic squash, Energen One-Cal drinks, Tab, Fresca, Diet Pepsi, Weight-watchers drinks and squash, PLJ (original and slightly sweetened), soda water, Vichy, Evian and Perrier water.

NOTE: No diabetic foods allowed apart from Boots Diabetic squash.

APPENDIX 3.2 DIARY INSTRUCTIONS

DIARY INSTRUCTIONS

Your eating diary contains one sheet for each day of the diet. A separate sheet should be used each day with the date and day of the week noted at the top.

SIDE 1

Please note down everything that you eat and drink, along with the time. Each item should be written down as soon as possible after it has been eaten. (It is easy to lose track of what you have had if you do not do this, so you will need to carry the diary around with you). You can use your diary to help you to keep a check on what you are eating.

SIDE 2

On this side of each page is a series of simple rating scales. These are completed by either circling a number or drawing a line on the scale to indicate your answer on a scale of 0 - 100.

For example:

If you were asked to rate how much you enjoyed your last visit to the dentist, you might have found it particularly unpleasant, so therefore you should circle 90 or 100. If you find it difficult to decide which number to choose, then it may be helpful to compare it with other days, or in this example, visits.

You will also notice that there is a separate sheet of rating scales for the pre- and post-diet weeks, These, again, should be complete on a daily basis during these two weeks.

At the end of each dat, please check that you have remembered to complete all parts of the diary.

APPENDIX 3.3 DAILY VISUAL ANALOGUE SCALES OF EATING DISTURBANCE

PLEASE EITHER PLACE A MARK ON THE LINE OR CIRCLE THE NUMBER WHICH BEST INDICATES HOW YOU HAVE BEEN FEELING TODAY

I have been preoccupied with thoughts of food

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have had strong urges to eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

Once I have started to eat, I have felt unable to stop

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have been afraid of losing control of my eating

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have eaten at times when I was not hungry

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have had to resist the urge to over-eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

APPENDIX 3.4 ADAPTED EATING DISORDERS EXAMINATION INTERVIEW

Q* I would like to ask you about any episodes of overeating that you have had over the past week.

OBJECTIVE BULIMIC EPISODE/OBJECTIVE OVEREATING

Q* Over the past week have there been any times when you have eaten a large amount of food? (at one go)

Q* Have there been any times when you have eaten what others might regard as a large amount of food?

Q Typically what have you eaten at these times?

Q If you were with others, how much did they eat?

Q Do you think that you could have resisted these episodes of eating (ie prevented them from occurring) or stopped them once they had started?

a) Objective bulimic episode

ie loss of control and large amount eaten.

Calculate number of occasions over last week.

Number of days where at least one event occurred ___
Number of episodes in total ___

Number of episodes outside meals ___

b) Objective overeating

ie no loss of control, but large amount eaten.

Number of days where at least one event occurred ___
Number of episodes in total ___

Number of episodes outside meals ___

INTERVIEW SUBJECTIVE BULIMIC EPISODES AND SUBJECTIVE OVEREATING

Q* Over the past week have there been any times when you have felt that you have eaten a large amount of food, but others might not agree? (by your standards - it's a subjective feeling)

NB eligible episodes must have been viewed as 'large'. If not skip to 'social eating'.

Q Typically what have you eaten at these times?

Q If you were with others, how much did they eat?

Q Do you think that you could have resisted these episodes of eating (ie prevented them from occurring) or stopped them once they had started? (could you have stopped if you had wanted to/ do you think you could have resisted in the first place - if you really had to?)

c) Subjective bulimic episodes

ie 'overeating' with loss of control, but where it is questionable whether the amount eaten would generally be regarded as large.

Number of days where at least one event occurred ___
Number of episodes in total ___

d) Subjective overeating

ie no loss of control, amount not large.

Number of days where at least one event occurred ___
Number of episodes in total ___

NATURE OF EPISODES

i) Nature of objective bulimic episodes

Duration

Q Over the past week, on average, how long have such episodes lasted?

Rate average duration in mins even if eating continued for several hours and there were periods where the subject was not eating.

Fullness

Q Have you felt full on such occasions? How full?

Were you distended? Was it uncomfortable or painful? Could you physically eat any more?

Rate on average how full the subject has felt afterwards.

0 - Overeaten, but has not felt full
1 -
2 - Overeaten until has felt slightly uncomfortable (bloated; physical sense of having overeaten)
3 -
4 - Overeaten until has felt moderately uncomfortable (definite distension, but not painful)
5 -
6 - Overeaten until it has been physically impossible to continue (painful severe abdominal distension)

ii) Nature of episodes of objective overeating (outside meals)

only for episodes of objective overeating outside meals.

Duration

Q Over the past week, on average, how long have such episodes lasted?

Rate average duration in mins even if eating continued for several hours and there were periods where the subject was not eating.

Fullness

Q Have you felt full on such occasions? How full?

Were you distended? Was it uncomfortable or painful?
Could you physically eat any more?

Rate on average how full the subject has felt afterwards.

0 - Overeaten, but has not felt full
1 -
2 - Overeaten until has felt slightly uncomfortable
(bloated; physical sense of having overeaten)
3 -
4 - Overeaten until has felt moderately uncomfortable
(definite distension, but not painful)
5 -
6 - Overeaten until it has been physically impossible to continue (painful severe abdominal distension)

APPENDIX 4.1 VISUAL ANALOGUE SCALES OF DISORDERED EATING

PLEASE EITHER PLACE A MARK ON THE LINE OR
CIRCLE THE NUMBER WHICH BEST INDICATES HOW
YOU FEEL RIGHT NOW

I am preoccupied with thoughts of food

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

Once I started to eat, I expect to feel unable to stop

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I am afraid of losing control of my eating

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I expect to have to resist the urge to over-eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

APPENDIX 4.1 continued VISUAL ANALOGUE SCALES OF DISORDERED EATING

PLEASE EITHER PLACE A MARK ON THE LINE OR
CIRCLE THE NUMBER WHICH BEST INDICATES HOW
YOU FEEL <u>RIGHT NOW</u>

I am preoccupied with thoughts of food

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

Once I had started to eat, I felt unable to stop

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

During the meal, I was afraid of losing control of my eating

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

During the meal, I had to resist the urge to over-eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

APPENDIX 5.1 RESTRAINT AND ATTITUDES TO BODY WEIGHT AND SHAPE - EDE-Q3

Below and over the page are some questions asking how you have been feeling about yourself and your weight. The questions are concerned with the PAST FOUR WEEKS ONLY (28 days).

Please read each question carefully and circle the appropriate number on the right. Please answer all the questions.

"ON HOW MANY DAYS OUT OF THE PAST 28 DAYS	No days	1-5 days	6-12 days	13-15 days	16-22 days	23-27 days	Every day
1. ....Have you been consciously trying to restrict the amount of food you eat to influence your shape or weight?	0	1	2	3	4	5	6
2. ....Have you gone for long periods of time (8 hours or more) without eating anything in order to influence your shape or weight?	0	1	2	3	4	5	6
3. ....Have you attempted to avoid eating any foods which you like in order to influence your shape or weight?	0	1	2	3	4	5	6
4. ....Have you attempted to follow definite rules regarding your eating in order to influence your shape or weight; for example, a calorie limit, a set amount of food, or rules about what or when you should eat?	0	1	2	3	4	5	6
5. ....Have you had a definite desire for your stomach to be flat?	0	1	2	3	4	5	6
6. ....Have you had a definite desire for your stomach to feel empty?	0	1	2	3	4	5	6
7. ....Has thinking about shape or weight interfered with your ability to concentrate on things you are interested in; for example, read, watch television or follow a conversation?	0	1	2	3	4	5	6
8. ....Have you had a definite fear that you might gain weight or become fat?	0	1	2	3	4	5	6
9. ....Have you felt fat?	0	1	2	3	4	5	6
10. ....Have you had a strong desire to lose weight?	0	1	2	3	4	5	6

APPENDIX 5.1 continued RESTRAINT AND ATTITUDES TO BODY WEIGHT AND SHAPE - EDE-Q3

OVER THE PAST FOUR WEEKS (28 DAYS)....	NOT AT ALL		SLIGHTLY		MODERATELY		MARKEDLY
12. ....Has your weight influenced how you think about (judge) yourself as a person?	0	1	2	3	4	5	6
13. ....Has your shape influenced how you think about (judge) yourself as a person?	0	1	2	3	4	5	6
14. ....How much would it distress you if you had to weigh yourself once a week for the next four weeks?	0	1	2	3	4	5	6
15. ....How dissatisfied have you felt about your weight?	0	1	2	3	4	5	6
16. ....How dissatisfied have you felt about your shape?	0	1	2	3	4	5	6
17. ....How thin have you wanted to be?	0	1	2	3	4	5	6
18. ....How uncomfortable have you felt seeing your body; for example, in the mirror in shop window reflections, while undressing or taking a bath or shower: (Only circle 4, 5 or 6 if you have avoided some occasions).	0	1	2	3	4	5	6
19. ....How uncomfortable have you felt about others seeing your body; for example, in communal changing rooms, when swimming or wearing tight clothes? (Only circle 4, 5 or 6 if you have avoided some occasions).	0	1	2	3	4	5	6

20. What would be the smallest weight gain, over a period of one week, which would definitely upset you? (Please circle the appropriate number).

7 pounds gain
6 pounds gain
5 pounds gain
4 pounds gain
3 pounds gain
2 pounds gain
1 pound gain

APPENDIX 5.2 VISUAL ANALOGUE SCALES OF DISORDERED EATING - DAILY RATINGS FOR DIARIES

PLEASE EITHER PLACE A MARK ON THE LINE OR CIRCLE THE NUMBER WHICH BEST INDICATES HOW YOU HAVE BEEN FEELING TODAY

I have been preoccupied with thoughts of food

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have had strong urges to eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

Once I have started to eat, I have felt unable to stop

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have been afraid of losing control of my eating

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have eaten at times when I was not hungry

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have had to resist the urge to over-eat

0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

I have felt fat

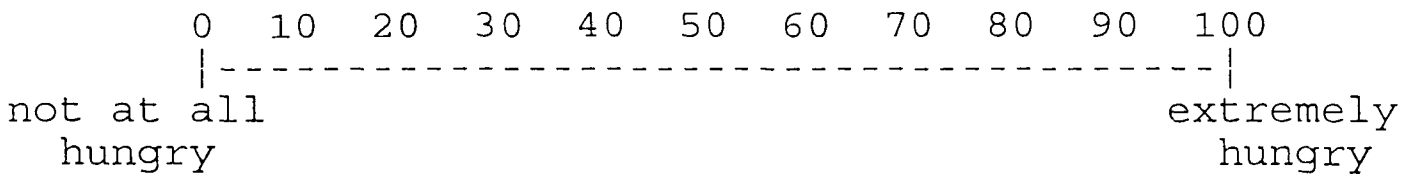
0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

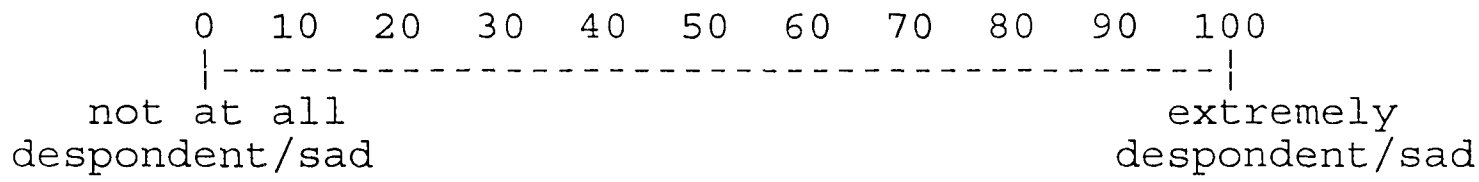
I have had cravings for carbohydrate foods

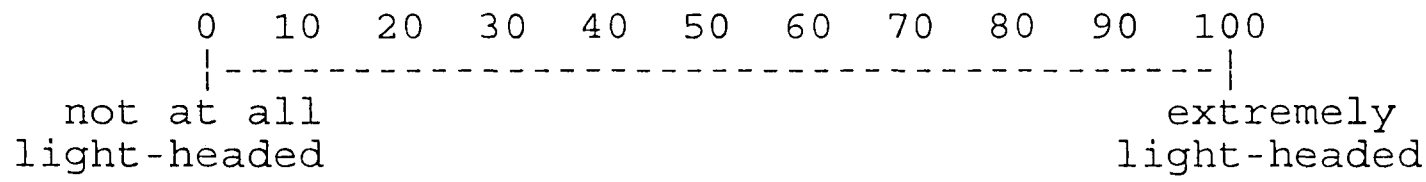
0 10 20 30 40 50 60 70 80 90 100
|----------|
not at all a great deal

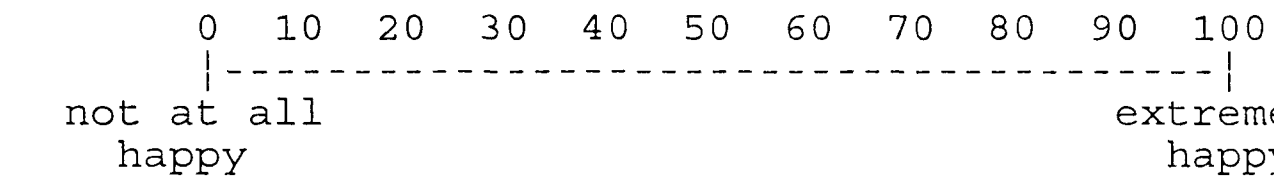
APPENDIX 6.1 VISUAL ANALOGUE SCALES - MOOD AND APPETITE

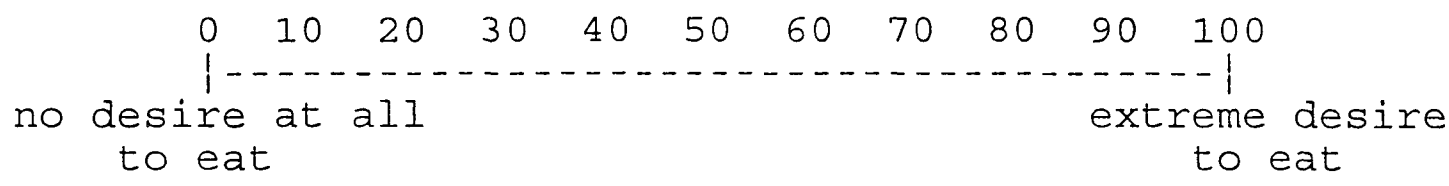
PLEASE EITHER PLACE A MARK ON THE LINE OR
CIRCLE THE NUMBER WHICH BEST INDICATES HOW
YOU FEEL <u>RIGHT NOW</u>

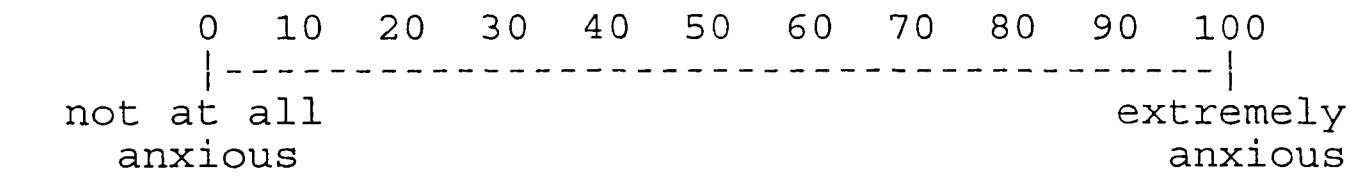


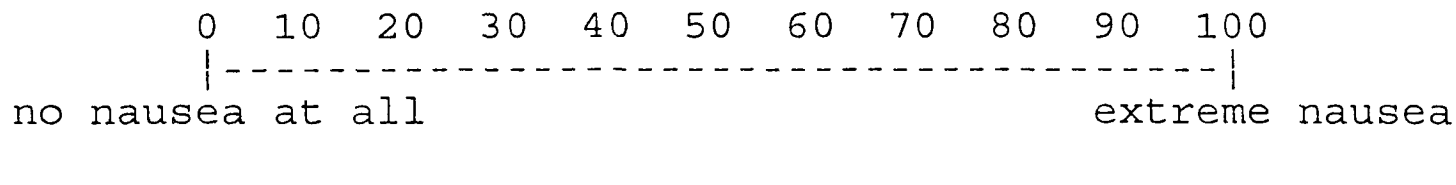












APPENDIX 7 APPENDICES OF TABLES, CHAPTERS 3 TO 7

TABLE 3.3: MEAN WEIGHT (±SD)		
	DIET MEAN (±SD)	CONTROL MEAN (±SD)
Weight 1 (baseline)	66.60 (±5.80)	66.73 (±8.89)
Weight 2 (baseline)	66.81 (±6.24)	66.61 (±8.96)
Weight 3 (diet)	65.32 (±6.10)	66.98 (±9.10)
Weight 4 (diet)	64.49 (±6.34)	66.55 (±8.85)
Weight 5 (diet)	63.30 (±6.32)	66.63 (±9.01)
Weight 6 (post-diet)	64.54 (±6.73)	66.64 (±8.68)

TABLE 3.4: PLASMA TOTAL AND FREE TRYPTOPHAN LEVELS: MEAN (±SEM)		
GROUP	DIET	CONTROL
	MEAN (±SEM)	MEAN (±SEM)
Plasma Total Tryptophan		
Baseline	10.92 (±0.47)	11.63 (±0.22)
End pre-diet week	10.49 (±0.36)	11.05 (±0.28)
End diet week 1	9.48 (±0.55)	11.23 (±0.42)
End diet week 2	9.50 (±0.42)	12.16 (±0.53)
End diet week 3	9.79 (±0.38)	11.01 (±0.50)
Plasma Free Tryptophan		
Baseline	0.99 (±0.09)	0.85 (±0.05)
End pre-diet week	0.88 (±0.05)	0.70 (±0.04)
End diet week 1	0.89 (±0.08)	0.76 (±0.04)
End diet week 2	0.93 (±0.08)	0.88 (±0.07)
End diet week 3	0.83 (±0.06)	0.77 (±0.08)

TABLE 3.5: APPETITE MEASURES (ANOVA)		
MEASURE	MAIN EFFECT (GROUP)	GROUP x TIME INTERACTION (GG)
VAS Hunger	$F_{1,29}$=1.47 p>0.05	$F_{27,809}$=1.40 p>0.05
TEST MEAL VAS:-		
Hunger	$F_{1,28}$=0.91 p>0.05	$F_{2,58}$=0.19 p>0.05
Wish to eat	$F_{1,29}$=0.14 p>0.05	$F_{2,60}$=0.1 p>0.05
Feel full	$F_{1,29}$=1.79 p>0.05	$F_{2,60}$=0.2 p>0.05
Find meal satisfying	$F_{1,29}$=0.79 p>0.05	$F_{2,60}$=1.08 p>0.05
Could eat a large amount of food	$F_{1,29}$=0.48 p>0.05	$F_{2,60}$=0.12 p>0.05
MACRONUTRIENT SELECTION:-		
Test meal kcal intake TOTAL	$F_{1,29}$=2.23 p>0.05	
CHO	$F_{1,29}$=1.32 p>0.05	
PRO	$F_{1,29}$=2.77 p>0.05	
FAT	$F_{1,29}$=2.25 p>0.05	
Test meal food preference qu'aire CHO	$F_{1,28}$=0.12 p>0.05	$F_{2,58}$=2.71 p=0.09
PRO	$F_{1,28}$=0.00 p>0.05	$F_{2,58}$=0.09 p>0.05
FAT	$F_{1,28}$=0.08 p>0.05	$F_{2,58}$=0.23 p>0.05
PRO vs CHO	$F_{1,25}$=6.45 p<0.01	$F_{2,52}$=0.43 p>0.05

TABLE 3.6: TEST MEAL CALORIE CONSUMPTION (MEAN ±SD)				
	DIET MEAN (±SD)		CONTROL MEAN (±SD)	
MEASURE	TEST MEAL 1	TEST MEAL 2	TEST MEAL 1	TEST MEAL 2
Carbohydrate	1599 (±628)	1576 (±562)	1474 (±716)	1640 (±585)
Protein	598 (±230)	551 (±181)	500 (±185)	586 (±176)
Fat	1702 (±693)	1440 (±679)	1312 (±554)	1656 (±454)
Total	3898 (±1471)	3566 (±1282)	3286 (±1304)	3757 (±1053)

TABLE 3.7: TEST MEAL FOOD PREFERENCE QUESTIONNAIRE - FREE AND FORCED CHOICE (MEAN ±SD)

	DIET MEAN (±SD)		CONTROL MEAN (±SD)	
MEASURE	TEST MEAL 1	TEST MEAL 2	TEST MEAL 1	TEST MEAL 2
Carbohydrate (pre meal)	5.0 (±2.0)	4.81 (±1.47)	4.67 (±2.09)	5.13 (±1.84)
(post meal)	0.25 (±0.45)	0.88 (±1.41)	0.73 (±0.96)	0.13 (±0.35)
Protein (pre meal)	3.19 (±1.97)	3.13 (±1.89)	2.73 (±2.05)	3.13 (±2.23)
(post meal)	0.31 (±0.79)	0.25 (±0.58)	0.27 (±0.70)	0.0 (±0.0)
Fat (pre meal)	4.88 (±1.86)	4.75 (±2.14)	4.53 (±1.25)	5.0 (±1.65)
(post meal)	1.0 (±1.26)	1.0 (±1.37)	1.0 (±1.2)	0.73 (±1.16)
PRO vs CHO (pre meal)	13.5 (±6.15)	13.43 (±6.37)	10.71 (±5.28)	14.14 (±6.64)
(post meal)	16.64 (±5.36)	17.93 (±5.17)	18.64 (±6.34)	20.64 (±5.31)

TABLE 3.8: EATING DISTURBANCE (ANOVA)		
MEASURE	MAIN EFFECT (GROUP)	GROUP x TIME INTERACTION (GG)
DIARY PRE- AND POST-DIET VAS:-		
Preoccupied with thoughts of food	$F_{1,29}$=0.89 p>0.05	$F_{6,179}$=1.53 p>0.05
Urge to eat	$F_{1,29}$=3.1 p=0.09	$F_{2,179}$=0.51 p>0.05
Fear loss of control	$F_{1,29}$=6.95 p<0.05	$F_{6,179}$=0.62 p>0.05
Unable to stop	$F_{1,29}$=5.67 p<0.05	$F_{6,179}$=0.53 p>0.05
Eaten when not hungry	$F_{1,29}$=1.86 p>0.05	$F_{6,179}$=0.38 p>0.05
Resist the urge to overeat	$F_{1,29}$=3.75 p=0.06	$F_{6,179}$=0.32 p>0.05
TEST MEAL VAS:-		
Preoccupied with thoughts of food	$F_{1,27}$=0.45 p>0.05	$F_{2,56}$=0.11 p>0.05
Fear loss of control	$F_{1,27}$=1.46 p>0.05	$F_{2,56}$=0.2 p>0.05
Unable to stop	$F_{1,27}$=2.71 p>0.05	$F_{2,56}$=0.58 p>0.05
Resist the urge to overeat	$F_{1,27}$=0.2 p>0.05	$F_{2,56}$=0.1 p>0.05

TABLE 3.9: EATING DISTURBANCE DIARY VAS RATINGS (MEAN ±SD)				
	DIET MEAN (±SD)		CONTROL MEAN (±SD)	
MEASURE	PRE-DIET	POST-DIET	PRE-DIET	POST-DIET
Preoccupied with thoughts of food	11.54 (±12.24)	24.67 (±17.26)	14.36 (±12.04)	21.46 (±14.16)
Urge to eat	14.70 (±12.71)	24.99 (±22.42)	19.10 (±14.98)	20.54 (±14.72)
Fear loss control	8.18 (±8.20)	17.27 (±17.95)	6.16 (±8.30)	4.44 (±6.48)
Unable to stop	9.27 (±9.14)	17.60 (±21.23)	9.46 (±10.76)	7.41 (±9.24)
Eaten when not hungry	26.88 (±19.53)	26.20 (±19.8)	25.83 (±23.32)	18.75 (±14.11)
Resist urge to overeat	12.75 (±14.53)	19.75 (±20.62)	12.68 (±13.83)	9.90 (±10.13)

TABLE 3.10: DAILY RATINGS OF MOOD (ANOVA)		
MEASURE	GROUP	GROUP x TIME INTERACTION (GG)
Despondent/sad	$F_{1,29}$=0.88 p>0.05	$F_{27,809}$=0.64 p>0.05
Tense	$F_{1,29}$=0.57 p>0.05	$F_{27,809}$=1.07 p>0.05
Anxious	$F_{1,29}$=0.01 p>0.05	$F_{27,809}$=1.13 p>0.05
Irritable	$F_{1,29}$=0.28 p>0.05	$F_{27,809}$=1.20 p>0.05

TABLE 3.11: DAILY RATINGS OF VAS (MOOD AND HUNGER) (MEAN ±SD)					
	PRE-DIET WEEK	WEEK 1	WEEK 2	WEEK 3	POST-DIET WEEK
DIET GROUP					
Hunger	27.81 (±15.21)	39.81 (±17.28)	34.5 (±17.87)	33.18 (±21.67)	31.39 (±17.7)
Despondent/sad	10.03 (±9.40)	10.61 (±12.64)	15.14 (±17.54)	12.12 (±10.51)	11.61 (±12.89)
Tense	14.87 (±13.18)	12.81 (±12.03)	12.79 (±18.32)	10.55 (±10.46)	11.91 (±14.78)
Anxious	11.01 (±10.18)	8.68 (±8.77)	12.63 (±15.49)	11.21 (±9.05)	11.67 (±13.68)
Irritable	13.81 (±10.92)	15.13 (±11.46)	15.93 (±16.05)	18.86 (±20.67)	15.18 (±14.07)
CONTROL GROUP					
Hunger	31.01 (±17.67)	31.86 (±17.77)	28.61 (±19.99)	32.68 (±22.27)	33.79 (±20.36)
Despondent/sad	17.89 (±18.73)	16.82 (±16.28)	13.55 (±12.51)	11.45 (±11.82)	11.79 (±10.67)
Tense	16.61 (±13.81)	19.98 (±16.52)	16.47 (±14.31)	10.94 (±11.47)	14.53 (±14.60)
Anxious	16.34 (±18.39)	16.84 (±16.91)	13.63 (±11.96)	11.30 (±12.26)	13.46 (±10.49)
Irritable	15.74 (±14.28)	19.15 (±15.55)	17.02 (±12.84)	10.66 (±10.19)	13.70 (±13.58)

TABLE 4.1: MEAN (±SEM): PLASMA TOTAL AND FREE TRYPTOPHAN						
	PLACEBO		T-		B	
MEAN (±SEM) (μg/ml)	Baseline	+4.5 hrs	Baseline	+4.5 hrs	Baseline	+4.5 hrs
Total Tryptophan	12.98 (±0.63)	9.93 (±0.63)	13.26 (±0.75)	2.89 (±0.27)	13.08 (±0.72)	15.42 (±1.48)
Free Tryptophan	0.84 (±0.08)	0.64 (±0.05)	0.91 (±0.09)	0.31 (±0.03)	0.88 (±0.09)	0.97 (±0.08)

TABLE 4.2: EFFECTS OF TRYPTOPHAN DEPLETION ON MACRONUTRIENT AND CALORIE INTAKE (ANOVA)	
MEASURE	MAIN EFFECT OF CONDITION
Carbohydrate	$F_{2,20}$=0.35, p>0.05
Protein	$F_{2,20}$=0.6, p>0.05
Fat	$F_{2,20}$=2.37, p>0.05
Total	$F_{2,20}$=1.67, p>0.05

TABLE 4.3: EFFECTS OF TRYPTOPHAN DEPLETION ON MEASURES OF APPETITE (ANOVA)			
MEASURE	MAIN EFFECT OF CONDITION (GG)	MAIN EFFECT OF TIME (GG)	CONDITION BY TIME INTERACTION (GG)
VAS Hunger	$F_{2,20}$=8.52 p<0.005	$F_{6,60}$=37.7 p<0.00001	$F_{12,120}$=2.16 p=0.09
VAS Wish to Eat	$F_{2,20}$=0.82 p>0.05	$F_{1,10}$=132.3 p<0.00001	$F_{2,20}$=3.26 p=0.06
VAS Feel Full	$F_{2,20}$=1.68 p>0.05	$F_{1,10}$=220.4 p<0.00001	$F_{2,20}$=1.55 p>0.05
VAS Find Meal Satisfying	$F_{2,20}$=0.66 p>0.05	$F_{1,10}$=8.69 p<0.01	$F_{2,20}$=1.83 p>0.05
Food Preference Qu'aire CHO	$F_{2,20}$=2.21 p>0.05	$F_{2,20}$=41.25 p<0.00001	$F_{4,40}$=1.68 p>0.05
PRO	$F_{2,20}$=0.19 p>0.05	$F_{2,20}$=17.91 p<0.0005	$F_{4,40}$=1.07 p>0.05
FAT	$F_{2,20}$=6.80 p<0.05	$F_{2,20}$=50.93 p<0.00001	$F_{4,40}$=1.54 p>0.05
PRO vs CHO	$F_{2,16}$=0.01 p>0.05	$F_{2,16}$=26.28 p<0.00001	$F_{4,32}$=0.21 p>0.05

TABLE 4.4: MEANS: EFFECTS OF TRYPTOPHAN DEPLETION ON TEST MEAL CALORIE CONSUMPTION			
	MEAN KJ (±SD)		
MEASURE	PLACEBO	T-	BALANCED
CHO	1839 (±578)	1907 (±647)	1848 (±615)
PRO	648 (±220)	688 (±167)	649 (±194)
FAT	1824 (±613)	2075 (±589)	1899 (±640)
TOTAL	4445 (±1338)	4663 (±1306)	4395 (±1342)

TABLE 4.5: MEANS: EFFECTS OF TRYPTOPHAN DEPLETION ON TEST MEAL FOOD PREFERENCE QUESTIONNAIRE

	MEAN (±SD)		
MEASURE	PLACEBO	T-	BALANCED
Carbohydrate			
baseline	4.17 (±2.58)	3.83 (±3.03)	3.67 (±2.26)
pre meal	5.75 (±1.91)	5.25 (±2.14)	4.75 (±2.09)
post meal	0.08 (±0.29)	0.50 (±0.90)	0.25 (±0.45)
Protein			
baseline	2.08 (±2.63)	2.50 (±2.97)	1.75 (±2.49)
pre meal	3.91 (±2.47)	3.58 (±2.81)	3.91 (±2.64)
post meal	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)
Fat			
baseline	3.92 (±2.68)	3.33 (±2.69)	2.67 (±2.30)
pre meal	6.08 (±2.19)	6.33 (±1.78)	4.83 (±2.66)
post meal	0.58 (±0.67)	1.00 (±1.13)	1.00 (±0.52)
PRO vs CHO			
baseline	13.90 (±7.27)	13.20 (±8.02)	13.4 (±6.75)
pre meal	9.18 (±4.47)	10.58 (±4.32)	10.91 (±5.16)
post meal	20.72 (±3.66)	21.42 (±3.31)	21.08 (±3.87)

TABLE 4.6: MEANS: EFFECTS OF TRYPTOPHAN DEPLETION ON APPETITE VAS RATINGS				
		MEAN (±SD)		
MEASURE VAS	TIME	PLACEBO	T-	BALANCED
Hunger	0 hours	50.42 (±26.5)	50.83 (±29.1)	33.3 (±27.7)
	+60 mins	48.5 (±23.8)	40.0 (±23.4)	24.17 (±24.3)
	+120 mins	53.3 (±27.1)	47.5 (±23.8)	34.17 (±24.7)
	+180 mins	65.0 (±19.3)	54.2 (±23.5)	40.8 (±23.9)
	+240 mins	69.6 (±18.1)	61.66 (±22.9)	55.0 (±25.7)
	pre meal	75.0 (±16.8)	67.1 (±21.4)	60.8 (±25.7)
	post meal	0.00 (±0.00)	1.67 (±3.9)	2.5 (±4.5)
Wish to Eat	pre meal	81.3 (±17.1)	70.83 (±17.3)	68.8 (±22.4)
	post meal	2.9 (±5.4)	5.8 (±7.9)	3.8 (±6.4)
Feel Full	pre meal	9.58 (±10.1)	9.17 (±10.0)	17.9 (±19.7)
	post meal	90.0 (±8.5)	85.8 (±14.4)	87.9 (±10.8)
Find Meal Satisfying	pre meal	72.1 (±23.7)	77.5 (±17.1)	74.2 (±23.5)
	post meal	91.3 (±8.0)	90.0 (±8.5)	80.0 (±23.4)

TABLE 4.7: EFFECTS OF TRYPTOPHAN DEPLETION ON MEASURES OF MOOD (ANOVA)			
MEASURE	MAIN EFFECT OF CONDITION (GG)	MAIN EFFECT OF TIME (GG)	CONDITION BY TIME INTERACTION (GG)
VAS Despondent/sad	$F_{2,20}$=0.38 p>0.005	$F_{6,60}$=4.58 p<0.05	$F_{12,120}$=0.87 p>0.05
VAS Tense	$F_{2,20}$=4.31 p=0.06	$F_{6,60}$=5.13 p<0.01	$F_{12,120}$=3.43 p<0.05
VAS Anxious	$F_{2,20}$=4.03 p=0.07	$F_{6,60}$=4.15 p<0.05	$F_{12,120}$=3.44 p<0.05
VAS Irritable	$F_{2,20}$=4.95 p<0.05	$F_{6,60}$=2.54 p>0.05	$F_{12,120}$=0.92 p>0.05
POMS Depression	$F_{2,20}$=2.21 p>0.05	$F_{2,20}$=41.25 p<0.00001	$F_{4,40}$=1.68 p>0.05
POMS Anxiety/Tension	$F_{2,20}$=0.19, p>0.05	$F_{2,20}$=17.91 p<0.0005	$F_{4,40}$=1.07 p>0.05

TABLE 4.8: MEANS: EFFECTS OF TRYPTOPHAN DEPLETION ON POMS DEPRESSION RATINGS

MEASURE	TIME	MEAN (±SD)		
		PLACEBO	T-	BALANCED
POMS Depression	Baseline (0 hours)	3.08 (±3.9)	1.08 (±1.8)	1.92 (±3.2)
	Pre meal (+4.5 hrs)	2.33 (±3.6)	0.92 (±2.0)	2.25 (±3.1)
	Post meal	0.9 (±1.5)	0.58 (±1.44)	1.33 (±2.5)

TABLE 4.9: MEANS: EFFECTS OF TRYPTOPHAN DEPLETION ON VAS AND POMS RATINGS OF MOOD

MEASURE		MEAN (±SD)		
		PLACEBO	T-	BALANCED
VAS Despondent/Sad	baseline +4.5 hrs	10.8 (±4.16) 6.7 (±2.57)	15.0 (±4.99) 9.2 (±4.5)	11.7 (±4.73) 8.3 (±3.87)
VAS Tense	baseline +4.5 hrs	27.9 (±6.15) 10.0 (±2.74)	10.8 (±4.16) 5.0 (±1.93)	11.7 (±5.48) 4.2 (±1.93
VAS Anxious	baseline +4.5 hrs	21.0 (±5.4) 7.5 (±3.29)	8.3 (±2.97) 4.2 (±1.93)	8.3 (±5.05) 4.2 (±1.93)
VAS Irritable	baseline +4.5 hrs	11.7 (±3.87) 6.25 (2.22)	5.0 (±2.31) 2.5 (±1.79)	7.5 (±4.10) 4.2 (±1.93)
POMS Anxiety/Tension	baseline +4.5 hrs	6.9 (5.06) 6.3 (±4.06)	4.4 (±3.57) 4.1 (±3.81)	3.9 (±3.06) 4.2 (±4.36)

TABLE 5.2: PLASMA TOTAL AND FREE TRYPTOPHAN LEVELS: MEANS (±SEM)			
GROUP		NORMAL CONTROL	BULIMIC
	TIME (HRS)	MEAN (±SEM)	MEAN (±SEM)
Plasma Total Tryptophan			
PLACEBO	0	12.98 (±0.63)	10.51 (±0.79)
	+4.5	9.93 (±0.63)	9.35*** (±0.88)
T-	0	13.25 (±0.75)	9.91 (±0.49)
	+4.5	2.89 (±0.27)	2.01*** (±0.12)
BALANCED	0	13.08 (±0.71)	10.48 (±0.57)
	+4.5	15.41 (±1.48)	10.98 (±0.82)
Plasma Free Tryptophan			
PLACEBO	0	0.84 (±0.07)	0.79 (±0.07)
	+4.5	0.63 (±0.05)	0.76* (±0.07)
T-	0	0.92 (±0.09)	0.82 (±0.06)
	+4.5	0.31 (±0.03)	0.33*** (±0.04)
BALANCED	0	0.88 (±0.09)	0.76 (±0.05)
	+4.5	0.97 (±0.08)	0.90** (±0.08)

*p<0.05, **p<0.005, ***p<0.0001 significantly different from baseline, all subjects pooled

TABLE 5.3: BULIMIC VS CONTROL: APPETITE MEASURES (ANOVA)			
MEASURE	MAIN EFFECT (GROUP)	MAIN EFFECT (CONDITION) (GG)	GROUP x CONDITION INTERACTION (GG)
VAS Hunger	$F_{1,16}$=1.10 p>0.05	$F_{2,32}$=9.74 p=0.0005	$F_{2,32}$=2.57 p=0.09
TEST MEAL VAS:-			
Wish to eat	$F_{1,16}$=0.92 p>0.05	$F_{2,32}$=5.61 p=0.01	$F_{2,32}$=0.93 p>0.05
Feel full	$F_{1,16}$=1.17 p>0.05	$F_{2,32}$=0.46 p>0.05	$F_{2,32}$=0.76 p>0.05
Find meal satisfying	$F_{1,16}$=22.43 p<0.0005	$F_{2,32}$=1.41 p>0.05	$F_{2,32}$=0.59 p>0.05
Could eat a large amount of food	$F_{1,16}$=1.01 p>0.05	$F_{2,32}$=0.99 p>0.05	$F_{2,32}$=0.43 p>0.05
MACRONUTRIENT SELECTION:-			
Test meal KJ intake TOTAL	$F_{1,16}$=4.75 p<0.05	F2$_{,32}$=4.15 p<0.05	$F_{2,32}$=3.0 P=0.08
CHO	$F_{1,16}$=3.91 p=0.07	$F_{2,32}$=0.21 p>0.05	$F_{2,32}$=1.35 P>0.05
PRO	$F_{1,16}$=3.06 p>0.05	$F_{2,32}$=1.63 p>0.05	$F_{2,32}$=1.46 P>0.05
FAT	$F_{1,16}$=4.60 p<0.05	$F_{2,32}$=4.99 p<0.05	$F_{2,32}$=2.69 p=0.09
Test meal food preference qu'aire CHO	$F_{1,16}$=1.14 p>0.05	F2$_{,32}$=0.27 p>0.05	$F_{2,32}$=0.55 p>0.05
PRO	$F_{1,16}$=1.04 p>0.05	F2$_{,32}$=1.55 p>0.05	$F_{2,32}$=0.67 p>0.05
FAT	$F_{1,16}$=3.41 p=0.08	F2$_{,32}$=0.79 p>0.05	$F_{2,32}$=4.67 p<0.05
PRO vs CHO*	$F_{1,12}$=5.51 p<0.01	F2$_{,24}$=0.78 p>0.05	$F_{2,24}$=0.65 p>0.05

*= degrees of freedom reduced because of missing data

TABLE 5.4: MEANS: APPETITE VAS RATINGS

		NORMAL CONTROL GROUP MEAN (±SD)			BULIMIC GROUP MEAN (±SD)		
MEASURE VAS	TIME	PLACEBO	T-	BALANCED	PLACEBO	T-	BALANCED
Hunger	0 hours	50.42 (±26.5)	50.83 (±29.1)	33.3 (±27.7)	40.0 (±36.3)	30.0 (±27.3)	34.6 (±29.6)
	+60 mins	48.5 (±23.8)	40.0 (±23.4)	24.2 (±24.3)	38.1 (±25.1)	20.0 (±19.3)	24.4 (±31.1)
	+120 mins	53.3 (±27.1)	47.5 (±23.8)	34.2 (±24.7)	51.3 (±25.9)	21.6 (±18.1)	29.4 (±27.3)
	+180 mins	65.0 (±19.3)	54.2 (±23.5)	40.8 (±23.9)	60.0 (±28.3)	27.0 (±22.2)	28.1 (±23.6)
	+240 mins	69.6 (±18.1)	61.7 (±22.9)	55.0 (±25.7)	63.8 (±28.8)	34.4 (±23.5)	46.9 (±24.6)
	pre meal	75.0 (±16.8)	67.1 (±21.4)	60.8 (±25.7)	68.8 (±24.2)	46.8 (±18.2)	45.9 (±33.4)
	post meal	0.00 (±0.00)	1.67 (±3.9)	2.5 (±4.5)	6.3 (±14.1)	5.0 (±9.3)	6.3 (±11.9)
Wish to Eat	pre meal	81.3 (±17.1)	70.8 (±17.3)	68.8 (±22.4)	66.3 (±30.2)	50.6**(±20.1)	52.9* (±27.7)
	post meal	2.9 (±5.4)	5.8 (±7.9)	3.8 (±6.4)	13.8 (±25.0)	7.5 (±11.6)	7.5 (±8.9)
Feel Full	pre meal	9.58 (±10.1)	9.2 (±10.0)	17.9 (±19.7)	11.3 (±12.5)	21.3 (±21.0)	21.3 (±20.3)
	post meal	90.0 (±8.5)	85.8 (±14.4)	87.9 (±10.8)	80.0 (±15.1)	73.8 (±22.6)	75.0 (±20.7)
Find Meal Satisfying	pre meal	72.1 (±23.7)	77.5 (±17.1)	74.2 (±23.5)	56.3 (±22.0)	54.0 (±14.0)	57.9 (±18.6)
	post meal	91.3 (±8.0)	90.0 (±8.5)	80.0 (±23.4)	76.3 (±19.2)	60.4 (±21.7)	52.5 (±24.9)
Could Eat Large Amount of Food	pre meal	79.2 (±20.1)	70.0 (±16.5)	67.9 (±16.7)	51.3# (±25.9)	44.0##(±20.2)	52.9 (±29.7)
	post meal	5.4 (±7.8)	10.0 (±11.3)	9.2 (±10.0)	21.3 (±27.5)	20.3 (±14.9)	15.0 (±12.0)

*p<0.05, **p<0.005, significantly different from placebo, all subjects pooled.
#p<0.01, ##p<0.005 BN subjects significantly different from controls.

TABLE 5.5: MEANS: BULIMIC VS CONTROL: TEST MEAL FOOD PREFERENCE QUESTIONNAIRE

	NORMAL CONTROL GROUP MEAN (±SD)			BULIMIC GROUP MEAN (±SD)		
MEASURE	PLACEBO	T-	BALANCED	PLACEBO	T-	BALANCED
Carbohydrate (pre meal)	5.75 (±1.91)	5.25 (±2.14)	4.75 (±2.09)	4.25 (±2.71)	4.38 (±2.45)	4.13 (±3.00)
(post meal)	0.08 (±0.29)	0.50 (±0.90)	0.25 (±0.45)	0.75 (±1.16)	0.88 (±1.46)	0.75 (±0.71)
Protein (pre meal)	3.91 (±2.47)	3.58 (±2.81)	3.91 (±2.64)	3.63 (±2.56)	3.13 (±2.90)	3.00 (±2.67)
(post meal)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.38 (±1.06)	0.38 (±1.06)	0.0 (±0.0)
Fat (pre meal)	6.08 (±2.19)	6.33 (±1.78)	4.83 (±2.66)	4.12 (±2.64)	4.0 (±2.62)	4.38 (±3.02)
(post meal)	0.58 (±0.67)	1.00 (±1.13)	1.00 (±0.52)	0.88 (±0.99)	0.62 (±0.92)	0.75 (±1.04)
PRO vs CHO (pre meal)	9.18 (±4.47)	10.58 (±4.32)	10.91 (±5.16)	7.38 (±5.90)	8.0 (±7.84)	8.38 (±7.82)
(post meal)	20.72 (±3.66)	21.42 (±3.31)	21.08 (±3.87)	13.25 (±9.48)	13.63 (±7.98)	14.5 (±8.91)

TABLE 5.6: MEANS: BULIMIC VS CONTROL: TEST MEAL CALORIE CONSUMPTION						
	MEAN KJ (±SD)					
MEASURE	NORMAL CONTROL GROUP			BULIMIC GROUP		
	PLACEBO	T-	BALANCED	PLACEBO	T-	BALANCED
CHO	1839 (±578)	1907 (±647)	1848 (±615)	1221 (±630)	1295 (±583)	1457 (±645)
PRO	648 (±220)	688 (±167)	649 (±194)	478 (±232)	573 (±159)	555 (±197)
FAT	1824 (±613)	2075 (±589)	1899 (±640)	1087 (±541)	1438*(±374)	1621 (±834)
TOTAL	4345 (±1338)	4663 (±1306)	4395 (±1342)	2786 (±1304)	3227 (±848)	3632 (±1515)

*p<0.05 significantly different from placebo, all subjects pooled.

TABLE 5.7: VAS RATINGS OF DISORDERED EATING: BULIMIC VS CONTROL (ANOVA)			
MEASURE	MAIN EFFECT OF GROUP	MAIN EFFECT OF CONDITION (GG)	GROUP BY CONDITION INTERACTION (GG)
Preoccupied with Thoughts of Food	$F_{1,13}$=2.83 p>0.05	$F_{2,26}$=1.97 p>0.05	$F_{2,26}$=1.35 p>0.05
Unable to Stop Eating	$F_{1,13}$=4.12 p=0.06	$F_{2,26}$=0.28 p>0.05	$F_{2,26}$=2.14 p>0.05
Fear Loss of Control	$F_{1,13}$=10.33 p<0.001	$F_{2,26}$=1.07 p>0.05	$F_{2,26}$=1.72 p>0.05
Resist the Urge to Overeat	$F_{1,13}$=4.92 p<0.05	$F_{2,26}$=0.13 p>0.05	$F_{2,26}$=0.29 p>0.05

TABLE 5.8: TABLE OF MEANS: VAS RATINGS OF DISORDERED EATING: BULIMIC VS CONTROL						
	NORMAL CONTROL GROUP MEAN (±SD)			BULIMIC GROUP MEAN (±SD)		
MEASURE	PLACEBO	T-	BALANCED	PLACEBO	T-	BALANCED
Preoccupied with thoughts of food pre	49.2 (±24.7)	31.9 (±24.5)	25.5 (±25.8)	44.3 (±21.5)	29.4 (±19.7)	33.1 (±26.0)
post	11.4 (±13.4)	8.3 (±19.9)	7.5 (±17.1)	13.8 (±20.0)	13.7 (±23.9)	20.0 (±23.3)
Unable to stop eating pre	27.9 (±25.0)	24.6 (±22.1)	19.1 (±23.9)	22.9 (±31.5)	23.1 (±28.9)	25.4 (±33.9)
post	23.0 (±23.7)	18.3 (±20.4)	12.5 (±16.0)	15.0 (±25.1)	23.1 (±31.5)	26.6 (±26.6)
Fear of loss of control pre	11.7 (±20.8)	18.6 (±16.9)	14.6 (±16.3)	11.4 (±21.9)	26.5 (±35.1)	25.3 (±31.2)
post	20.0 (±23.2)	15.0 (±20.2)	7.5 (±10.6)	22.5 (±34.1)	23.0 (±34.1)	21.9 (±30.9)
Resist urge to overeat pre	25.8 (±25.4)	27.5 (±20.9)	23.6 (±24.6)	32.9 (±29.8)	24.6 (±28.8)	27.9 (±34.3)
post	20.5 (±23.3)	15.4 (±15.3)	20.8 (±26.4)	26.3 (±32.0)	31.6 (±37.2)	29.1 (±32.6)

TABLE 5.9: VAS AND POMS RATINGS OF MOOD: BULIMIC VS CONTROL (ANOVA)			
MEASURE	MAIN EFFECT OF GROUP	MAIN EFFECT OF CONDITION (GG)	GROUP BY CONDITION INTERACTION (GG)
Despondent/sad	$F_{1,16}=5.85$ $p<0.05$	$F_{2,32}=0.20$ $p>0.05$	$F_{2,32}=1.01$ $p>0.05$
Tense	$F_{1,16}=4.53$ $p<0.05$	$F_{2,32}=2.32$ $p>0.05$	$F_{2,32}=1.09$ $p>0.05$
Anxious	$F_{1,16}=7.26$ $p<0.05$	$F_{2,32}=1.71$ $p>0.05$	$F_{2,32}=0.81$ $p>0.05$
Irritable	$F_{1,16}=7.37$ $p<0.05$	$F_{2,32}=2.42$ $p>0.05$	$F_{2,32}=0.16$ $p>0.05$
POMS depression*	$F_{1,13}=8.62$ $p=0.01$	$F_{2,26}=4.34$ $p<0.05$	$F_{2,24}=2.35$ $p>0.05$
POMS anxiety/tension*	$F_{1,13}=5.78$ $p<0.05$	$F_{2,26}=2.33$ $p>0.05$	$F_{2,26}=0.19$ $p>0.05$

*= degrees of freedom reduced because of missing data

TABLE 5.10: GROUP MEANS: VAS AND POMS RATINGS OF MOOD		
MEASURE	MEAN (±SD)	
	NORMAL CONTROL GROUP	BULIMIC GROUP
VAS Despondent/Sad	17.7 (±11.1)	15.7 (±13.5)
VAS Tense	8.2 (±10.7)	13.6 (±11.94)
VAS Anxious	7.1 (±9.1)	11.0 (±12.7)
VAS Irritable	5.2 (±8.35)	15.0 (±15.2)
POMS Anxiety/Tension	4.6 (±3.7)	7.5 (±4.4)

TABLE 5.11: MEANS: POMS DEPRESSION RATINGS							
MEASURE	TIME	NORMAL CONTROL GROUP MEAN (±SD)			BULIMIC GROUP MEAN (±SD)		
		PLACEBO	T-	BALANCED	PLACEBO	T-	BALANCED
POMS Depression	Baseline (0 hours)	3.08 (±3.9)	1.08 (±1.8)	1.92 (±3.2)	10.50 (±10.4)	7.13 (±6.9)	6.50 (±6.6)
	Pre meal (+4.5 hrs)	2.33 (±3.6)	0.92 (±2.0)	2.25 (±3.1)	7.50 (±7.2)	5.25 (±6.7)	6.0 (±7.3)
	Post meal	0.9 (±1.5)	0.58 (±1.44)	1.33 (±2.5)	10.28 (±11.8)	6.63 (±7.9)	7.0 (±9.2)

TABLE 5.12: DIARY VAS AND POMS RATINGS OF MOOD: BULIMIC GROUP (ANOVA)			
MEASURE	MAIN EFFECT OF CONDITION (GG)	MAIN EFFECT OF TIME (GG)	CONDITION BY TIME INTERACTION (GG)
Despondent/sad	$F_{2,6}$=2.19 p>0.05	$F_{6,18}$=1.45 p>0.05	$F_{12,36}$=0.43 p>0.05
Tense	$F_{2,6}$=0.81 p>0.05	$F_{6,18}$=2.20 p>0.05	$F_{12,36}$=0.83 p>0.05
Anxious	$F_{2,6}$=2.11 p>0.05	$F_{6,18}$=2.01 p>0.05	$F_{12,36}$=1.57 p>0.05
Irritable	$F_{2,6}$=1.55 p>0.05	$F_{6,18}$=2.51 p>0.05	$F_{12,36}$=1.52 p>0.05
POMS depression	$F_{2,8}$=1.11 p>0.05	$F_{1,4}$=1.61 p<0.05	$F_{2,8}$=0.77 p>0.05
POMS anxiety/tension	$F_{2,8}$=1.13 p>0.05	$F_{1,4}$=0.23 p>0.05	$F_{2,8}$=5.68 p<0.05
BDI	$F_{2,10}$=0.28 p>0.05		

TABLE 5.13: MEANS (±SD) FOR BULIMIC GROUP: DIARY VAS, BDI AND POMS RATINGS OF MOOD			
MEASURE	MEAN (±SD)		
	PLACEBO	T-	BALANCED
VAS Despondent/Sad	31.4 (±19.4)	15.7 (±11.6)	28.6 (±20.0)
VAS Tense	28.3 (±22.6)	18.6 (±14.7)	35.1 (±18.9)
VAS Anxious	29.1 (±22.8)	20.1 (±12.4)	34.9 (±19.8)
VAS Irritable	28.6 (±18.1)	18.0 (±11.1)	25.9 (±11.4)
POMS Depression	10.5 (±8.3)	7.7 (±3.8)	10.4 (±4.4)
POMS Anxiety/Tension	10.2 (±6.7)	10.1 (±2.4)	7.5 (±1.2)
BDI	12.4 (±19.3)	10.6 (±10.5)	15.7 (±20.9)

TABLE 5.14: DIARY VAS RATINGS OF EATING DISTURBANCE: BULIMIC GROUP (ANOVA)			
MEASURE	MAIN EFFECT OF CONDITION (GG)	MAIN EFFECT OF TIME (GG)	CONDITION BY TIME INTERACTION (GG)
Hunger	$F_{2,6}$=0.37 p>0.05	$F_{6,18}$=2.45 p>0.05	$F_{12,36}$=0.27 p>0.05
Preoccupied with Thoughts of Food	$F_{2,8}$=0.79 p>0.05	$F_{6,24}$=0.74 p>0.05	$F_{12,48}$=0.49 p>0.05
Strong Urges to Eat	$F_{2,8}$=0.51 p>0.05	$F_{6,24}$=0.36 p>0.05	$F_{12,48}$=1.21 p>0.05
Felt Unable to Stop Eating	$F_{2,8}$=0.66 p>0.05	$F_{6,24}$=0.73 p>0.05	$F_{12,48}$=1.03 p>0.05
Fear of Loss of Control	$F_{2,8}$=5.68 p=0.07	$F_{6,24}$=0.45 p>0.05	$F_{12,48}$=0.77 p>0.05
Eaten when Not Hungry	$F_{2,8}$=0.11 p>0.05	$F_{6,24}$=0.33 p>0.05	$F_{12,48}$=1.11 p>0.05
Resist Urge to Overeat	$F_{2,8}$=0.12 p>0.05	$F_{6,24}$=0.69 p>0.05	$F_{12,48}$=0.76 p>0.05
Felt Fat	$F_{2,8}$=4.16 p>0.05	$F_{6,24}$=1.18 p>0.05	$F_{12,48}$=0.77 p>0.05
Carbohydrate Cravings	$F_{2,8}$=2.35 p>0.05	$F_{6,24}$=0.33 p>0.05	$F_{12,48}$=0.68 p>0.05

TABLE 5.15: MEANS (±SD) FOR BULIMIC GROUP: DIARY VAS RATINGS OF EATING DISTURBANCE			
MEASURE	MEAN (±SD)		
	PLACEBO	T-	BALANCED
Hunger	33.4 (±13.4)	30.6 (±20.0)	29.4 (±14.9)
Preoccupied with Thoughts of Food	24.9 (±21.9)	20.1 (±19.4)	26.4 (±14.3)
Strong Urges to Eat	28.0 (±19.4)	21.3 (±20.1)	24.3 (±18.4)
Felt Unable to Stop	18.3 (±17.3)	16.4 (±18.5)	20.3 (±16.9)
Fear of Loss of Control	21.6 (±20.4)	18.3 (±20.9)	26.1 (±21.9)
Eaten when not Hungry	15.9 (±17.5)	16.9 (±18.7)	18.3 (±17.6)
Resist Urge to Overeat	19.9 (±22.6)	19.3 (±21.2)	21.1 (±18.4)
Felt Fat	24.0 (±22.5)	24.7 (±25.5)	29.5 (±20.2)
Carbohydrate Cravings	32.6 (±23.1)	22.1 (±21.4)	30.8 (±18.8)

TABLE 6.1: PLASMA PROLACTIN PRESENTED AS MEAN CHANGE FROM BASELINE (mU/L) (±SD)			
TIME	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
30 mins	-30.1 (±72.4)	-49.4 (±127.2)	-40.6 (±53.1)
60 mins	-47.1 (±53.6)	-68.3 (±72.0)	-65.9 (±48.2)
90 mins	-37.0 (±44.5)	-65.4 (±47.9)	-80.3 (±41.6)
120 mins	-16.8 (±42.9)	-39.9 (±48.5)	-101.8 (±32.8)
150 mins	18.4 (±61.8)	-4.8 (±66.0)	-103.3 (±26.0)
180 mins	21.2 (±64.6)	12.9 (±98.1)	-105.1 (±31.6)

TABLE 6.2: ORAL TEMPERATURE EXPRESSED AS MEAN CHANGE FROM BASELINE (^{O}C) (±SD)			
TIME	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
30 mins	0.03 (±0.14)	-0.02 (±0.13)	-0.07 (±0.17)
60 mins	0.09 (±0.21)	-0.02 (±0.14)	-0.08 (±0.25)
90 mins	0.08 (±0.17)	0.02 (±0.22)	-0.05 (±0.27)
120 mins	0.17 (±0.22)	0.05 (±0.21)	-0.02 (±0.21)
150 mins	0.23 (±0.16)	0.11 (±0.16)	-0.05 (±0.24)
180 mins	0.24 (±0.18)	0.13 (±0.28)	-0.04 (±0.19)

TABLE 6.3: TEST MEAL CALORIE CONSUMPTION (KJ) (MEAN ±SD)			
	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
Total	3440 (±1101)	3123 (±1223)	3456 (±1126)
Protein	752 (±617)	578 (±237)	629 (±263)
Carbohydrate	1590 (±528)	1496 (±671)	1710 (±630)
Fat	1240 (±450)	1049 (±540)	1117 (±367)

TABLE 6.4: VAS RATINGS OF HUNGER (MEAN ±SD)			
TIME	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
0 mins	39.5 (±18.8)	38.8 (±19.6)	36.6 (±17.7)
30 mins	38.2 (±21.1)	43.3 (±25.5)	38.0 (±16.6)
60 mins	34.5 (±21.2)	37.3 (±23.4)	39.5 (±18.7)
90 mins	43.4 (±19.1)	36.7 (±25.2)	43.5 (±22.8)
120 mins	48.5 (±19.3)	39.1 (±25.4)	45.9 (±23.9)
150 mins	52.2 (±21.2)	46.8 (±26.2)	50.8 (±21.5)
180 mins	49.5 (±20.7)	51.6 (±25.3)	56.8 (±23.7)
PRE MEAL	53.3 (±18.9)	57.1 (±20.7)	60.8 (±19.7)
POST MEAL	5.8 (±8.9)	9.4 (±10.1)	6.3 (±11.1)

TABLE 6.5: VAS RATINGS OF ANXIETY (MEAN ±SD)			
TIME	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
0 mins	25.4 (±18.1)	21.2 (±20.7)	19.2 (±18.2)
30 mins	14.7 (±12.7)	19.6 (±19.6)	15.5 (±16.8)
60 mins	17.5 (±18.0)	19.8 (±18.5)	13.2 (±14.7)
90 mins	13.2 (±12.6)	18.1 (±18.6)	12.5 (±14.9)
120 mins	13.3 (±15.2)	15.7 (±15.0)	12.9 (±16.6)
150 mins	13.6 (±13.8)	18.8 (±24.1)	13.1 (±15.0)
180 mins	12.3 (±15.1)	18.8 (±26.6)	11.6 (±13.8)
PRE MEAL	14.5 (±19.6)	15.0 (±16.2)	15.8 (±15.8)
POST MEAL	13.1 (±16.7)	12.3 (±12.7)	6.6 (±8.5)

TABLE 6.6: VAS RATINGS OF NAUSEA (MEAN ±SD)			
TIME	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
0 mins	6.9 (±12.2)	9.9 (±16.5)	7.3 (±12.6)
30 mins	7.8 (±12.7)	13.2 (±17.9)	8.2 (±11.9)
60 mins	14.5 (±25.6)	13.6 (±17.2)	7.1 (±8.7)
90 mins	12.3 (±17.2)	10.7 (±15.1)	4.0 (±4.4)
120 mins	7.4 (±8.6)	9.4 (±12.9)	4.1 (±4.6)
150 mins	8.4 (±12.5)	11.2 (±15.5)	6.5 (±8.0)
180 mins	7.7 (±10.4)	6.8 (±7.32)	6.2 (±7.8)
PRE MEAL	10.9 (±18.2)	6.4 (±9.7)	6.5 (±8.6)
POST MEAL	15.3 (±22.9)	6.5 (±8.5)	1.3 (±3.1)

TABLE 6.7: EFFECTS OF ORAL MCPP ON PRE AND POST MEAL VAS RATINGS			
	MCPP (15MG)	MCPP (7.5MG)	PLACEBO
Meal Satisfaction			
pre	64.9 (±12.1)	67.0 (±19.6)	65.7 (±16.5)
post	75.4 (±20.6)	73.9 (±17.2)	82.6 (±15.5)
Feel Full			
pre	18.6 (±15.0)	20.1 (±12.7)	21.2 (±17.5)
post	79.6 (±13.2)	81.0 (±13.1)	79.0 (±18.6)
Desire to Eat			
pre	58.9 (±14.7)	59.2 (±16.5)	60.7 (±17.8)
post	9.1 (±7.6)	13.1 (±12.2)	9.4 (±8.3)

TABLE 6.8: EFFECTS OF ORAL MCPP ON VAS RATINGS OF MOOD AND NAUSEA (ANOVA)			
	DRUG	TIME	DRUG BY TIME INTERACTION
Anxious	$F_{2,22}$=2.23 p>0.05	$F_{6,66}$=0.68 p>0.05	$F_{12,132}$=0.43 p>0.05
Happy	$F_{2,22}$=0.69 p>0.05	$F_{6,66}$=4.14 p<0.001	$F_{12,132}$=0.69 p>0.05
Despondent/sad	$F_{2,22}$=0.94 p>0.05	$F_{6,66}$=2.48 p<0.05	$F_{12,132}$=0.97 p>0.05
Nausea	$F_{2,22}$=0.46 p>0.05	$F_{6,66}$=1.18 p>0.05	$F_{12,132}$=1.28 p>0.05

TABLE 6.9: PLASMA LEVELS OF MCPP (MEAN ±SD)		
TIME	MCPP (15MG)	MCPP (7.5MG)
0 mins	0.00 (±0.00)	0.00 (±0.00)
30 mins	0.21 (±0.50)	0.00 (±0.00)
60 mins	2.28 (±2.57)	4.26 (±9.59)
90 mins	4.93 (±7.35)	6.13 (±11.21)
120 mins	8.49 (±8.54)	7.07 (±11.32)
150 mins	10.85 (±12.48)	6.96 (±10.22)
180 mins	12.38 (±18.13)	5.96 (±10.18)

TABLE 7.1: VAS RATINGS OF HUNGER: MEAN (±SD)

CONDITION	AMPHETAMINE	AMPH-GR68755	GR68755	PLACEBO
0 min	40.2 (±14.0)	40.5 (±13.3)	41.6 (±13.6)	41.9 (±15.3)
20 min	43.0 (±15.0)	42.2 (±13.2)	43.4 (±14.3)	43.6 (±15.5)
40 min	42.3 (±16.1)	40.5 (±15.4)	44.9 (±14.1)	44.6 (±15.6)
60 min	35.4 (±15.6)	37.8 (±17.9)	46.4 (±14.1)	45.6 (±14.3)
80 min	30.8 (±18.9)	36.9 (±21.9)	46.7 (±14.5)	44.1 (±15.8)
100 min	31.5 (±18.7)	35.4 (±22.7)	48.9 (±13.1)	45.3 (±16.1)
120 min	32.1 (±18.9)	36.9 (±23.4)	49.6 (±12.2)	47.1 (±16.2)
140 min	34.5 (±19.3)	36.9 (±25.8)	51.4 (±13.3)	48.3 (±17.3)
160 min	33.6 (±18.5)	38.7 (±24.1)	52.6 (±13.6)	51.2 (±17.1)
180 min	33.8 (±17.3)	41.1 (±24.0)	54.6 (±12.6)	51.4 (±16.5)

TABLE 7.2: VAS RATINGS OF DESIRE TO EAT: MEAN (±SD)

CONDITION	AMPHETAMINE	AMPH-GR68755	GR68755	PLACEBO
0 min	35.9 (±16.0)	35.5 (±14.5)	38.2 (±16.4)	37.1 (±18.1)
20 min	38.2 (±16.6)	37.9 (±15.9)	40.2 (±17.2)	39.6 (±19.8)
40 min	37.8 (±18.4)	32.9 (±16.6)	42.1 (±19.5)	40.1 (±18.5)
60 min	29.8 (±15.7)	29.4 (±16.8)	42.1 (±18.0)	40.2 (±18.2)
80 min	25.4 (±17.2)	28.7 (±20.0)	43.6 (±16.9)	40.6 (±19.6)
100 min	25.2 (±16.7)	26.8 (±22.0)	45.1 (±17.4)	41.1 (±20.3)
120 min	25.0 (±17.6)	28.7 (±22.1)	44.9 (±16.2)	43.6 (±19.1)
140 min	26.2 (±18.0)	31.8 (±22.6)	48.1 (±18.0)	44.2 (±19.8)
160 min	28.1 (±18.6)	30.1 (±25.4)	48.6 (±18.1)	46.1 (±21.3)
180 min	27.9 (±17.7)	30.1 (±23.8)	50.3 (±17.1)	49.3 (±22.0)

TABLE 7.3: MEAN (±SD): EFFECT OF AMPHETAMINE AND GR68755 ON VAS RATINGS OF SATIETY

VAS MEASURE	PLACEBO	GR68755	AMPHETAMINE	AMPH-GR68755
Found meal satisfying	70.0 (±12.0)	66.0 (±11.9)	52.1 (±20.0)	51.2 (±19.7)
Feel full	77.3 (±14.7)	72.0 (±11.5)	68.5 (±13.4)	72.0 (±11.5)

TABLE 7.4: MEAN (±SD): EFFECTS OF AMPHETAMINE AND GR68755 CALORIE AND MACRONUTRIENT INTAKE				
CALORIE INTAKE (KJ)	PLACEBO	GR68755	AMPHETAMINE	AMPH-GR68755
Protein	999.5 (±304.9)	1044.5 (±393.9)	671.4 (±375.8)	664.3 (±415.2)
Carbohydrate	2435.5 (±892.4)	2263.7 (±703.5)	1741.3 (±977.4)	1713.0 (±862.1)
Fat	2329.7 (±671.8)	2431.0 (±797.1)	1599.6 (±967.4)	1450.1 (±985.2)
Total	5715.7 (±1567.0)	5920.1 (±1758.3)	3930.0 (±2168.4)	3827.7 (±2115.1)

TABLE 7.5: EFFECTS OF AMPHETAMINE AND GR68755 ON RATINGS OF MACRONUTRIENT PREFERENCE (ANOVA)			
ANOVA	CARBOHYDRATE	PROTEIN	FAT
Time	$F_{1,21}$=54.92 p<0.001	$F_{1,21}$=99.48 p<0.001	$F_{1,21}$=51.57 p<0.001
Amphetamine	$F_{1,21}$=23.8 p<0.001	$F_{1,21}$=24.2 p<0.001	$F_{1,21}$=16.44 p<0.001
GR68755	$F_{1,21}$=0.41 p>0.05	$F_{1,21}$=0.4 p>0.05	$F_{1,21}$=0.39 p>0.05
Time x amphetamine	$F_{1,21}$=14.11 p<0.01	$F_{1,21}$=15.55 p<0.001	$F_{1,21}$=8.97 p<0.01
Time x GR68755	$F_{1,21}$=1.58 p>0.05	$F_{1,21}$=0.39 p>0.05	$F_{1,21}$=1.15 p>0.05
Amph x GR68755	$F_{1,21}$=3.18 p>0.05	$F_{1,21}$=2.59 p>0.05	$F_{1,21}$=0.3 p>0.05
Time x AMPH x GR68755	$F_{1,21}$=0.18 p>0.05	$F_{1,21}$=1.61 p>0.05	$F_{1,21}$=0.65 p>0.05

TABLE 7.6: MEAN (±SD): FORCED AND FREE CHOICE MACRONUTRIENT SELECTION				
	PLACEBO	GR68755	AMPHETAMINE	AMPH-GR68755
Forced PRO vs CHO pre	17.0 (±5.1)	17.5 (±5.8)	12.8 (±6.7)	14.5 (±6.5)
post	7.5 (±6.6)	7.1 (±6.0)	6.4 (±6.3)	7.0 (±6.0)
Carbohydrate pre	4.4 (±2.0)	4.3 (±2.4)	2.5 (±1.9)	2.2 (±1.7)
post	0.6 (±1.0)	1.0 (±1.5)	0.7 (±1.1)	0.6 (±0.95)
Protein pre	4.7 (±1.9)	4.9 (±1.8)	2.9 (±2.0)	2.5 (±2.2)
post	0.3 (±0.6)	0.5 (±1.2)	0.1 (±0.4)	0.2 (±0.7)
Fat pre	5.0 (±2.0)	5.1 (±2.2)	3.5 (±2.2)	3.0 (±2.3)
post	1.6 (±1.3)	1.5 (±1.5)	1.2 (±1.2)	1.3 (±1.8)

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Effect of acute tryptophan depletion on mood and appetite in healthy female volunteers

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Twelve healthy female subjects received the following three drinks in a double-blind, semi-balanced, cross-over design: (a) 50 g of amino acids without L-tryptophan (LTP); (b) 50 g of amino acid with LTP (balanced); (c) plain water. Compared to both the balanced amino acid mixture and plain water, the LTP drink significantly lowered plasma total and free tryptophan at 4.5 h. However, compared to the two control conditions, there was no effect of the LTP drink on subjective ratings of mood or hunger. Similarly, the LTP drink did not alter significantly either total calorie intake or the macronutrient content of a test meal 5 h after drink ingestion.

Key words: L-tryptophan; appetite; hunger; mood; calories; macronutrient

Introduction

Brain 5-hydroxytryptamine (5-HT) pathways influence several important behaviours in animals and humans. There is much evidence, for example, that brain 5-HT neurones are involved in the regulation of mood, with numerous studies indicating that patients with depressive disorders have impaired brain 5-HT function; in addition, drugs that are helpful in treating depression often facilitate 5-HT neurotransmission (for reviews, see Cowen and Anderson, 1991; Deakin, 1991).

There is also strong evidence that 5-HT neurones are involved in the control of appetite, perhaps principally by influencing processes of satiety (Blundell and Hill, 1987). Drugs that increase brain 5-HT function decrease food intake in both animals and humans (Kennet and Curzon, 1991; Godall *et al.*, 1992) while the converse has been demonstrated for 5-HT receptor antagonists, though the latter effect depends to a large extent on the experimental conditions employed (Silverstone and Schuyler, 1975; Stallone and Nicolaidis, 1989). It has also been suggested that changes in brain 5-HT function may have consequences for macronutrition selection in that increasing brain 5-HT neurotransmission may tend selectively to reduce carbohydrate intake (Wurtman and Wurtman, 1979). This effect, however, has not been found in all studies.

The synthesis of 5-HT depends on the conversion of the amino acid precursor of 5-HT, L-tryptophan (LTP), to 5-hydroxytryptophan. This process is catalysed by tryptophan hydroxylase, an enzyme confined to 5-HT neurones (Lovenberg, Jequier and Sjoerdsma, 1968). Under normal physiological conditions, tryptophan hydroxylase is unsaturated with LTP; accordingly altering the availability of LTP to the brain changes brain 5-HT synthesis (Ashcroft, Eccleston and Crawford, 1965; Fernstrom and Wurtman, 1971). Administration of LTP to rats, for example, increases brain 5-HT synthesis and release (Gessa *et al.*, 1974; Morris *et al.*, 1987; Curzon, 1981; Sharp, Bramwell and Grahame-Smith, 1992).

Conversely, lowering the availability of LTP to the brain decreases 5-HT synthesis (Biggio *et al.*, 1974; Moja *et al.*, 1989). One way of achieving this effect is to administer a mixture of amino acids from which LTP has been omitted. This manoeuvre decreases the supply of LTP to the brain through two mechanisms: (a) by lowering plasma tryptophan concentration; and (b) by restricting brain entry of LTP through competition of the other amino acids for transport across the blood–brain barrier (Young *et al.*, 1985).

This method of lowering brain 5-HT function has been applied to both animals and humans. In rats, Biggio *et al.* (1974) demonstrated that an LTP-free mixture of essential amino acids decreased brain 5-HT turnover. In addition, we have shown recently that a similar amino acid treatment decreased the 5-HT release evoked by electrical stimulation of the dorsal raphe nucleus *in vivo* (Gartside, Cowen and Sharp, 1992). In humans, Young *et al.* (1985) reported that a 100 g LTP-free amino acid mixture markedly decreased plasma total and free LTP. This effect was associated with a small but significant lowering of mood in healthy male volunteers. In a subsequent study, Young *et al.* (1988) found that LTP depletion produced by the amino acid mixture resulted in a modest but significant decrease in protein intake in male subjects.

The purpose of the present study was to examine the effect of acute LTP depletion on mood, appetite and food intake in healthy female volunteers. Previous studies by ourselves and others indicate that females may be more vulnerable than males to the effects of LTP depletion on brain 5-HT function (Anderson *et al.*, 1990). We therefore predicted that LTP depletion would significantly lower mood and increase food intake in our subjects.

Methods

Volunteers

Twelve female volunteers between the ages of 19 and 27, and of normal weight for height (BMI range 17.4–29.0) were recruited

through advertisement. All volunteers were in good health, had no history of psychiatric disorder, no dietary restriction (two subjects were non-strict vegetarian) and no history of eating difficulties. The mean Beck Depression Inventory (BDI) score was 6.42 (range 0–17). All women had a regular menstrual cycle.

Experimental design

Subjects were required to attend the research unit for three complete mornings, each a week apart such that no session fell within the pre-menstrual period of their cycle (i.e. each session fell within days 3–12, with these days being confirmed before the test sessions). The three conditions consisted of the amino acid drink without tryptophan (T –), the balanced control drink (B) and a control session where subjects ingested a drink of water with no amino acids (placebo). Because of the consistency and taste of the amino acid mixture, it was necessary to use a semi-balanced design with placebo always administered first in order to ensure that the remaining two conditions remained double-blind. Order effects were therefore gauged on the basis of T – and B drinks, with the placebo condition used as an anchor for the group comparison.

Measures

During the test sessions, mood was measured using the Profile of Mood States Questionnaire (POMS), which had been amended to examine current mood, and 100 mm visual analogue scales (VAS). The parameters were despondent/sad, anxious, irritable and tense.

Appetite was measured using a free-choice, buffet-style test meal accompanied by VAS and food preference questionnaires before and after the meal. All food items were weighed to the nearest 0.1 g before and after the meal. The macronutrient and energy value of each meal was then calculated from these data using specifically designed software. Food preference questionnaires were those of Blundell and Hill (1987), and consisted of one free-choice and one forced-choice checklist. The latter examined the preference for carbohydrate over protein food items.

The way in which a typical meal was made up was as detailed in Table 1.

Dietary manipulation

The two amino acid mixtures were made up as drinks. The control drink contained 16 amino acids (52 g of amino acids in total), and was designed to be nutritionally balanced (B). The experimental drink was of the same composition with the exclusion of the 1.15 g of L-tryptophan (T –). Amino acid amounts were as follows: L-alanine, 2.75 g; L-arginine, 2.45 g; L-cysteine, 1.35 g; glycine, 1.6 g; L-histidine, 1.6 g; L-isoleucine, 4.0 g; L-leucine, 6.75 g; L-lysine monohydrochloride, 5.5 g; L-methionine, 1.5 g; L-phenylalanine, 2.85 g; L-proline, 6.1 g; L-serine, 3.45 g; L-threonine, 3.45 g; L-tyrosine, 3.45 g; L-valine, 4.45 g; L-tryptophan, 1.15 g. These are as described by Young *et al.* (1985), based on the ratios of amino acids found in human milk.

Amino acids were mixed with 300 ml refrigerated water, and the resulting suspension was flavoured with 12.5 mg saccharin and blackcurrant.

Procedure

Subjects arrived at 8.30 a.m. after an overnight fast, and spent the test session in a testing room. At 8.40, baseline VASs, POMS and food preferences were taken, followed by 10 ml fasting plasma. At 9.00 the drink was administered. Between 9.30 a.m. and 1.30 p.m., VASs were administered once per hour. At 1.30 rating scales were given followed by the second 10 ml blood sample (+4.5 h).

The test meal was presented at 1.40, and volunteers were left to determine the end of the meal. Rating scales were completed once the meal was over, and then volunteers were sent home with a booklet of rating scales to complete before and after their evening meal and lunch next day.

Biochemical measures

Blood samples (10 ml) were taken into heparin tubes prior to the drink ('0' min) and 4.5 h after the drink. Plasma samples were separated by centrifugation. Plasma total and free tryptophan were determined by fluorometric detection according to the method of Bloxam and Warren (1974) and Bloxam, Hutson and Curzon (1977).

Data analysis

All data were analysed by analysis of variance using the BMDP p2V statistical package or the ANOVA suite on Supastat (SPA). This analysis was group (order 1 versus order 2). Greenhouse Geisser probability was used where repeat measures were part of the analysis.

Table 1 Composition of test meal

	Choice range[a]	Quantity	Approx weight
Bread	wholemeal, white, soft grain	8 slices of one type	270 g
Margarine		Small tub	250 g
Sandwich filling	ham, tuna, chicken roll, cheddar cheese, cottage cheese, peanut butter, marmite, jam	4 choices (minimum of 2 to be protein)	e.g. 230 g tuna; 250 g cottage cheese; 100 g ham; 340 g peanut butter
Crisps	3 flavours	2 packets of one type	60 g
Pudding	yoghurt, rice pudding, fruit salad	1 type	e.g. 270 g yoghurt
Fruit	apple, orange, banana	2 items	e.g. 120 g apple; 100 g banana
Biscuits	digestive, rich tea, morning coffee	1 type	120 g
Chocolate	milk, plain, white	12 squares of one type	75 g
Tomatoes		2	140 g

[a]Choice determined by food preference ratings completed by volunteer prior to study.

Results

Biochemical measures

Plasma concentrations of total and free tryptophan (TRP) were analysed for all three conditions. For levels of total plasma TRP, the ANOVA yielded a significant main effect of treatment ($F_{2,22}=33.77$, $p<0.001$) and of time ($F_{1,11}=57.1$, $p<0.001$). There was also a significant treatment by time interaction ($F_{2,22}=45.13$, $p<0.001$). *Post hoc* t-tests revealed that there was a highly significant fall in total TRP levels in the (T−) condition ($p<0.0005$). This fall constituted a 78% decrease in plasma TRP across the 4.5 h. With the placebo drink condition there was also a significant fall of TRP levels with time ($p<0.0005$) although this was of a lesser magnitude of a 23% decrease across the 4.5 h. There was no significant difference between baseline and +4.5 h for the balanced drink condition (see Fig. 1).

There was also a significant main effect of treatment ($F_{2,22}=11.14$, $p<0.001$) and of time ($F_{1,11}=30.22$, $p<0.001$) for the analysis of plasma free TRP. The treatment by time interaction was also significant ($F_{2,22}=33.27$, $p<0.001$). The pattern of change in free TRP concentrations was very similar to that seen with total TRP concentrations; in the (T−) condition, levels fell by 67% ($p<0.0005$) across the 4.5 h compared with a 24% decline in the placebo condition ($p<0.001$). There was no significant difference between baseline and +4.5 h for the balanced drink condition. For all of these analyses there was no significant difference between the baseline measures (Fig. 2).

Mood measures

In these analyses the order effect did not approach significance (Fs <1.0). The ANOVA revealed no significant main effects of treatment for VAS ratings of anxiety ($F_{2,20}=4.03$, $p=0.066$), and tension ($F_{2,20}=4.31$, $p=0.06$), but revealed significant main effects of treatment for irritability ($F_{2,20}=4.95$, $p=0.035$) (see Table 2). There was a significant treatment by time interaction for anxiety ($F_{12,120}=3.44$, $p=0.019$) and for tension ($F_{12,120}=3.43$, $p=0.029$). However, further examination of these data revealed that these effects were a reflection of

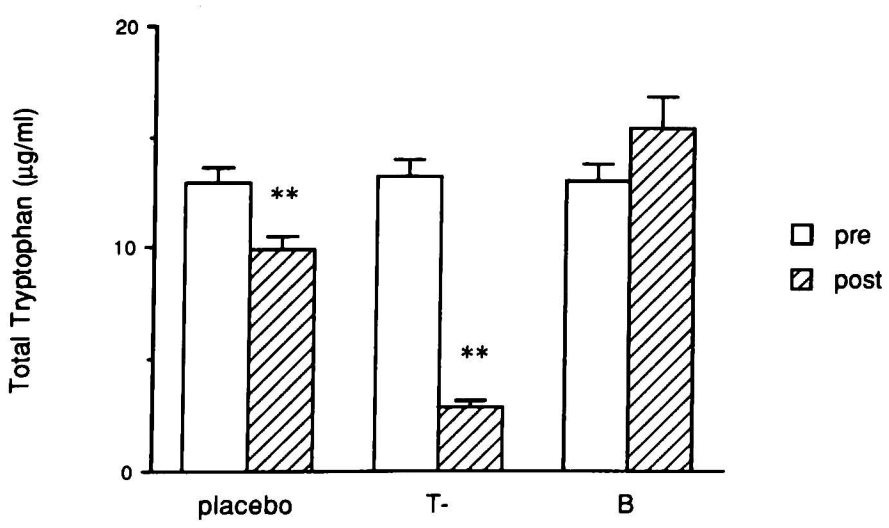


Figure 1 Mean (±SEM) plasma total tryptophan levels (µg/ml) at baseline and +4.5 h in 12 female subjects after (a) a tryptophan-depleted amino acid mixture (T−), (b) control mixture (B) and (c) inactive placebo. Total tryptophan levels fell significantly in the (T−) and placebo condition (analysis of variance). $^{**}p<0.0005$. All +4.5-h levels are significantly different from each other ($p<0.0005$) (*post hoc* paired t-test)

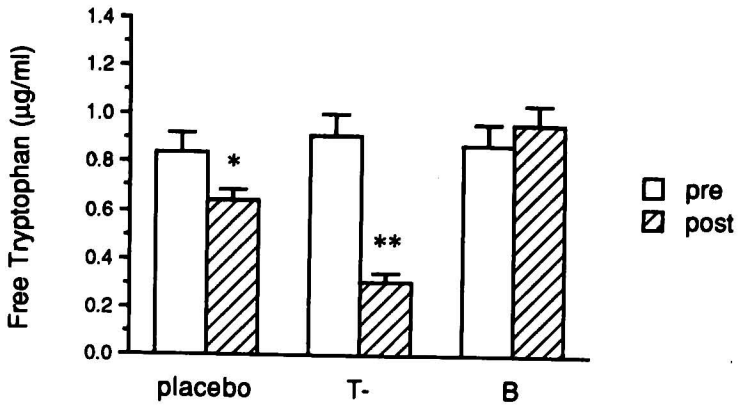


Figure 2 Mean (±SEM) plasma free tryptophan levels (µg/ml) at baseline and +4.5 h in 12 female subjects after (a) tryptophan-depleted amino acid mixture (T−), (b) control mixture (B) and (c) inactive placebo. Total tryptophan levels fell significantly in the (T−) and placebo condition (analysis of variance). $^{**}p<0.0005$, $^{*}p<0.001$. All +4.5-h levels are significantly different from each other ($p<0.0005$) (*post hoc* paired t-test)

increased scores in the initial placebo session, thus suggesting that the amino acid load had no impact on these scales (Table 2). The VAS measure of despondency revealed no significant main effect of treatment or of treatment by time interaction (for treatment $F_{2,20}=0.38$, $p=0.67$). This was confirmed by use of a standardized questionnaire, the POMS subscale for depression, which yielded similar results (for treatment $F_{2,16}=0.64$, $p=0.5$) (Table 2). An analysis of longer term changes in mood was also carried out. However, as a result of poor compliance with completion of VAS diaries, only six complete sets of VAS and POMS data were available for this analysis; in order to evaluate whether there was a longer term effect on mood, the POMS scores for test day evening and midday of the following day were analysed. The treatment effect was not significant ($F_{2,12}=1.82$, $p=0.2$). Treatment by time interaction was not significant ($F_{2,12}=0.73$, $p=0.50$) (data not shown). In addition to this, none of the VAS mood ratings approached significance, suggesting that the drink had no longer term impact on mood.

In order to assess whether there was any correlation between pre-treatment depression scores and effects of TRP depletion on mood, we correlated the BDI scores obtained at the screening interview with the change in mood scores obtained on the VAS and POMS. None of these correlations approached significance with the exception of the POMS depression ratings in the placebo condition (Pearson's $r=-0.51$, $p=0.05$), suggesting that the mood of subjects with high baseline scores correlated with a higher fall in POMS depression ratings across the initial test morning.

Appetite measures

In these analyses, the order effect did not approach significance (all F values <1). For VAS hunger ratings there was a significant main effect of treatment ($F_{2,20}=8.52$, $p=0.003$), but no interaction of treatment by time ($F_{12,120}=2.16$, $p=0.1$) (Table 2). The absence of this interaction suggests that the condition effect was not an effect of the amino acid manipulation. Inspection of the means indicated a baseline difference such that the balanced drink condition ratings were considerably lower than the other two conditions, and that the condition effect was merely a contamination of this difference throughout the test session.

Table 2 Effect of placebo, tryptophan-depleting amino acid mixture (T−) and balanced amino acid mixture (B) on VAS and POMS ratings

	(Placebo) Mean (±SEM)		(T−) Mean (±SEM)		(B) Mean (±SEM)	
VAS ratings	Baseline	+4.5 h	Baseline	+4.5 h	Baseline	+4.5 h
sad/despondent	10.8±4.16	6.7±2.57	15.0±4.99	9.2±4.5	11.7±4.73	8.3±3.87
anxious	21.0±5.40	7.5±3.29	8.3±2.97	4.2±1.93	8.3±5.05	4.2±1.93
tense	27.9±6.15	10.0±2.74	10.8±4.16	5.0±1.93	11.7±5.48	4.2±1.93
irritable	11.7±3.87	6.25±2.22	5.0±2.31	2.5±1.79	7.5±4.10	4.2±1.93
hungry	50.4±7.65	75.0±4.85	50.8±8.40	67.1±6.18	33.3±8.00	60.8±7.42
POMS depression	3.1±1.13	2.3±1.04	1.1±0.52	0.9±0.58	1.9±0.92	2.3±0.89

Table 3 Effect of placebo, tryptophan-depleting amino acid mixture (T−), and balanced amino acid mixture (B) on macronutrient and total kJ intake at test meal

kJ intake	Placebo Mean (±SEM)	(T−) Mean (±SEM)	(B) Mean (±SEM)
Protein	647.9±63.57	688.1±48.47	649.1±56.00
Carbohydrate	1839.8±166.94	1907.1±186.86	1847.9±177.56
Fat	1824.4±176.99	2074.8±170.06	1898.5±184.87
Total	4345.4±386.39	4663.9±377.09	4395.5±387.49

An analysis of the test meal revealed that there was no main effect of condition on total calorie intake ($F_{2,20}=1.67$, $p=0.22$), or on the intake of specific macronutrients; for total carbohydrate intake ($F_{2,20}=0.35$, $p=0.68$), protein intake ($F_{2,20}=0.6$, $p=0.54$) and for total fat intake ($F_{2,20}=2.37$, $p=0.13$) (see Table 3). Food preference questionnaire scores generally confirmed the above finding of no specificity in macronutrient selection between the three conditions both for forced- and free-choice selection. For forced-choice protein versus carbohydrates ($F_{2,16}=0.01$, $p=0.95$); for free-choice protein ($F_{2,20}=0.19$, $p=0.8$) and for free-choice carbohydrate ($F_{2,20}=2.21$, $p=0.14$). However, there was a significant condition effect for free-choice fat ratings on this scale ($F_{2,20}=6.80$, $p=0.0017$) but no treatment by time interaction ($F_{4,40}=1.54$, $p=0.23$). Further examination of the data revealed the effect to be a result of a lower baseline and pre-meal rating in the (B) condition (data not shown).

Discussion

In confirmation of earlier studies, we found that the LTP-free amino acid mixture produced striking decreases in plasma total and free LTP over the 4.5-h sampling period. In contrast to our hypotheses, however, we found no effect of the LTP-free mixture on mood or appetite compared either to the balanced LTP-containing mixture or to the placebo drink.

Our study differed in a number of respects from those of Young *et al.* (1985) in which significant changes were found in mood and food intake following acute LTP depletion. An important difference is that the 50 g dose of amino acid mixture that we employed is about half that used in the study of Young *et al.* (1985) in which a significant lowering of mood was found. We chose this dose because a pilot study using 100 g yielded an unacceptable incidence of nausea and vomiting. The 50 g drink was well tolerated and leads to a lowering in plasma LTP similar to the 100 g drink. However, a comparison of percentage change from baseline between the two doses yields very similar levels of change in the T− condition. We achieved a 78% decrease in total TRP compared with a decline of 76% in the Young *et al.* (1985) study. Similarly, plasma free TRP fell by 67% compared with Young *et al.*'s 60% decline. There were some differences in the (B) condition where we found no overall changes in both total and free TRP compared with Young *et al.* who had increases of 72% and 84% respectively. It is possible that this difference in the balanced conditions may have had a bearing on the psychological data collected; there may have been more of a contrast between the two conditions in the Young *et al.* study because of these differences in TRP levels. Another differing factor may be that, with a smaller dose of amino acids, the LTP that remains in plasma presumably has rather less competition from other amino acids for transport at the blood–brain barrier (see Introduction). It is possible, therefore, that the reduction in LTP availability to the brain produced by the 50 g drink may not have diminished brain 5-HT function sufficiently to produce a detectable lowering of mood. The 50 g dose, however, has been used in preference to the larger dose by research groups; for example, Abbott *et al.* (1992) have demonstrated that this dose blocks morphine analgesia.

Another important difference between these studies is that Young *et al.* (1985, 1987) studied male subjects while we studied females. However, we expected females, if anything, to be more vulnerable to the effects of LTP depletion on mood and appetite than males. We have previously found, for example, that while a calorie-restricting diet lowers plasma LTP concentrations in both males and females, only in females is this reduction in LTP availability associated with altered brain 5-HT function. Similarly Delgado *et al.* (1989) found that a diet that moderately restricted LTP intake (700 mg daily) altered brain 5-HT function in women but not men, while a more stringent reduction in dietary LTP intake (200 mg daily) altered brain 5-HT function in both men and women.

Variations in the menstrual cycle are known to alter mood and appetite (Goodall *et al.*, 1991; Bowen and Grunberg, 1990). It is possible, therefore, that the effect of LTP depletion in females could be confounded by variations in the menstrual cycle. However, we did not carry out tests in the pre-menstrual phase and there was no evidence of systematic bias in the allocation of particular drinks to a specific phase of the menstrual cycle.

An important factor in our demonstration of no effect of LTP depletion on mood may be the lack of vulnerability of the subjects we studied. The volunteers in our investigation were carefully screened and, in general, had very low depression scores prior to entry into the study. Young has commented that some of the subjects in the studies that showed a depressive

effect of LTP depletion had pre-treatment depression ratings in the high normal range, which may have increased their vulnerability to the mood-lowering effect of LTP depletion (Abbott *et al.*, 1992). This proposal is strengthened by the finding of Delgado *et al.* (1990) that the LTP-free amino acid drink produced an acute relapse in depressive symptomatology in patients recently recovered from a severe depressive episode. It is possible therefore that a depressive mood reaction to LTP depletion is an indication of a more general vulnerability to develop depressive symptomatology. However, we did not find any evidence from the present study that those subjects with higher BDI scores at entry were more vulnerable to the effects of TRP depletion on mood.

Young *et al.* (1988) have also reported that a 50 g LTP-free amino acid mixture produced a small but significant decline in protein selection in a buffet test meal. We did not demonstrate this effect. However, the effects of altering LTP availability on food intake and selection are subtle and depend strongly on the experimental conditions employed (Blundell, 1984). For example, the effects of LTP supplementation on subsequent macronutrient selection are dependent upon macronutrients administered with the LTP (Blundell and Hill, 1987); there may also be sex differences (Leiter, Hrboticky and Anderson, 1987). It could be argued that our lack of demonstration of an effect of tryptophan depletion on appetite was a result of the design which demanded that subjects fast overnight and for the entire morning. In these circumstances, a further increase in appetite and food intake might be hard to demonstrate. However, a previous study (Goodall and Silverstone, 1988) which also employed an overnight and morning fast found a significant enhancement of food intake by the selective 5-HT receptor antagonist metergoline, suggesting that it is possible to show increases in appetite following 5-HT receptor blockade even in fasted subjects. In addition, the study of Young *et al.* (1988), which found a decrease in the intake of protein at test meal, also employed an overnight and morning fast prior to the test meal.

In conclusion, our studies confirm that an LTP-free amino acid mixture reliably lowers plasma total and free LTP. However, in carefully screened healthy female subjects, there do not appear to be effects of this procedure on mood, appetite or food intake. Recent studies suggest that psychological effects of LTP depletion may be more striking in subjects with higher degrees of vulnerability to affective symptomatology (Miller *et al.*, 1992), and future studies investigating these groups are likely to be of great interest.

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