THE CONTRIBUTION OF CHOLECYSTOKININ TO APPETITE REGULATION IN PIGS WITH DIVERGENT GENETIC POTENTIAL FOR GROWTH AND FEED INTAKE

By

RUHONG JIANG

Bachelor of Science in Biological Science
Fudan University
Shanghai, China
1986

Master of Science in Reproductive Physiology
Beijing Agricultural University
Beijing, China
1991

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 1997

THE CONTRIBUTION OF CHOLECYSTOKININ TO APPETITE REGULATION IN PIGS WITH DIVERGENT GENETIC POTENTIAL FOR GROWTH AND FEED INTAKE

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEGEMENTS

I sincerely thank Dr. Archie C. Clutter for his advise and patience in the preparation of this thesis, as well as, throughout my graduate program. I am very thankful to committee member Dr. Joseph P. McCann for his guidance in establishing and validating the CCK-8 radioimmunoassay as well as the CCK-8 data analysis.

I would also like to thank Drs. Christa Hanson and Daniel Pomp for serving as members of my dissertation committee and their help in preparation of this thesis.

I would like to thank Dr. Rod Geisert and his graduate students for their assistance with animal cannulations. I gratefully acknowledge Dr. David Buchanan for establishing the selection lines, Dr. Jens Rehfeld for kindly providing the CCK antisera and Dr. Brain Kirkpatrick for serving as a collaborator in the study.

I would like to especially thank Mr. Shoji Sasaki for his invaluable friendship and assistance in the molecular genetics laboratory. Also a special thanks to Ms. Denise Dalton and Ms. Michelle Michelle for their assistance in the endocrinology laboratory. I also extend my appreciation to Mrs. Carol Bradley for her help in the preparation of this thesis.

I also would like to thank my parents for their support and encouragement throughout my entire education process. Most importantly, I acknowledge with deep gratitude, the support and understanding of my wife, Li Li.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Chapter II

Chapter III

Chapter IV

Chapter V

CHAPTER I

INTRODUCTION

Feed intake is one of the major factors that determine the profitability of livestock production. The physiological processes that result in hunger and satiety have been the focus of extensive research, but the specific differences in genotype which contribute to the variation in appetite in animal populations remain largely unknown. Genetic variation in the control of hunger and satiety originates from differences in genes that encode the proteins involved. Evaluation of candidate proteins in populations that differ only in their genetic propensity to eat may lead to the identification of the responsible genes and regulatory sequences.

Pigs have been a useful model species for studying appetite and related obesity (Anika et al., 1981; Houpt et al., 1979). Diverse selection lines of pigs have been studied to understand genetic variation in obesity (Wangsness et al., 1981). However, this approach has not been used to identify the relationship between genetic variation and mechanisms of appetite regulation. Our laboratory has lines of pigs from the same base population that have undergone ten generations of divergent selection for either fast (F) or slow (S) postweaning average daily gain (ADG). Woltmann et al. (1992) reported that the F and S lines differed in ADG (0.16 kg/d) and average daily feed intake (ADFI) (0.41 kg/d) when allowed ad libitum access to feed, but had similar ADG when feed intake was

standardized. Because these lines originated from a common base population and have been maintained in the same environment, the differences in appetite can be attributed to selection-induced changes in the frequencies of DNA sequences (gene alleles and gene regulatory sequences) that determine this trait. Thus, investigation of the genetic variation at the DNA level may help develop new methods to improve the genetic merit of this trait (e.g. molecular marker-assisted selection (MSA), Soller et al., 1982; Rothschild et al., 1990). MSA selection will first require identification of candidate genes or anonymous genetic markers associated with traits of interest. A few studies have shown association of genes with quantitative trait loci (QTL) in pigs (Rothschild et al., 1990; Jung et al., 1989; Clamp et al., 1992; Andersson et al., 1994; Yu et al., 1995).

The hormone cholecystokinin-8 (CCK-8) has been reported to play a role in appetite regulation through its ability to induce satiety in various species including pigs (Anika et al., 1981; Della-Fera et al., 1979; Micelli et al., 1983; McLaughlin et al., 1985; Denbow et al., 1982). Consequently, a study was conducted to determine the physiological and molecular bases for the genetic differences in feed intake between the F and S lines of pigs by focusing on CCK-8 as a candidate hormone. The objectives of the present study were (1) to determine if plasma concentrations of the satiety hormone CCK-8 differed between the lines during feeding; (2) to evaluate the dosage-dependent effects of exogenous CCK-8 on feed intake in pigs from F and S; (3) to estimate the relationship of CCK genotypes with ADG and backfat (BF) in offspring of F1 (F × S) sires.

CHAPTER II

REVIEW OF LITERATURE

I. Regulation of Food Intake

The regulation of food intake is a complex process which involves interactions between circulating nutrients, hormones and neurotransmitters (Baile et al., 1983; Morley, 1987, 1995; Woods and Gibbs, 1989). Early studies were directed at the potential ability of circulating nutrients (e.g. glucose, free fatty acids) to interact with either specific CNS neuronal populations (e.g. neurons in the hypothalamus) or with peripheral afferent neuronal pathways. Gibbs et al. (1973) reported that intraperitoneal injection of the intestinal peptide hormone cholecystokinin (CCK) reduced meal size in rats, thus beginning the "peptide revolution" in the study of the control of feeding. To date, many peptide hormones secreted by the gut and/or brain have been shown to have effect on feeding. Some of the candidate satiety peptides include CCK, bomebesin, somatostatin, glucagon and calcitonin. Among these peptides, however, the best-characterized satiety peptide is CCK, which is released from the duodenum during meals in many species and appears to effectively inhibit feeding whether administered peripherally via any of several routes or directly into CNS (Morley, 1987; Reidelberger, 1994). Another group of peptides, opioid peptides have been shown to stimulate food intake at both peripheral and central sites (Bechara et al., 1985). Extensive evidence has demonstrated that

neuropeptide Y (NPY) increases food intake when administered exogenously (Clark et al., 1984; Levine et al., 1984; Stanley et al., 1984; Stanley et al., 1986; Leibowitz, 1990; Akabayashi et al., 1994; Stanley et al., 1992) and it is suggested that NPY is a long-term hunger signal (Shibasaki et al., 1993; Billinton et al., 1991). Insulin action in the brain reduces food intake. The relationship between plasma and CNS insulin levels may change in association with appetite and obesity, indicating an important role of insulin in the central control of food intake and energy balance (Woods et al., 1976; Woods et al., 1979; Plata-Salaman et al., 1986; McGowan et al., 1992; Brief et al., 1994). The recent cloning and sequencing of the *ob* gene has suggested that its product, leptin, may regulate food intake and body weight (Zhang et al., 1994). A new model has been proposed that leptin, insulin and NPY may act as long-term regulators of food intake and body weight (Kaiyala et al., 1995; Figlewicz et al., 1996).

Hypothalamus and Food Intake

Food intake is regulated mainly by the CNS. The hypothalamus is an area of the brain associated with food intake. It receives and integrates internal and external signals, resulting in appropriate responses. Electrical stimulation of the lateral hypothalamus area (LH) initiates feeding, but electrical or chemical destruction of LH results in aphagia and weight loss (Hetherington et al., 1940). Electrical stimulation of the ventromedial hypothalamus (VMH) inhibits eating in hungry animals, but ablation of this area produces hyperphgia and obesity (Anand et al., 1951). Thus, interactions between the two areas may control food intake.

Opioid and Feeding

Opioid peptides refer to a group of chemicals that produce a morphine-like effect. At least 20 opioid peptides from brain, pituitary and adrenal tissue have been isolated and characterized. All of them have an amino-terminal Met or Leu-enkephalin sequence, and all are derived from one of three precursor molecules, pro-opiomelancortin (POMC), proenkephalin or prodynorphin.

Multiple opioid receptors exist in the brain and have been classified as Mu (μ) receptors, which have high affinity for morphine and β-endorphin; delta (δ) receptors, which have high affinity for enkephalin; and kappa (κ) receptors, which have high affinity for dynorphin. The pioneering study of Holtzman (1974) showed that naloxone was a highly specific antagonist for Mu opioid receptor and decreased food intake in rats. Subsequently, naloxone has been shown to decrease food intake in a variety of species, including rats and mice (Holtzman, 1974), sheep (Baile al., 1981; Alavis et al., 1991), guinea pigs (Billington et al.,1990), rabbits (Sanger et al., 1981), and cats (Foster et al., 1980). Among the three types of opioid receptors, κ and μ receptors appear to be most important in the hyperphagia response (Morley et al., 1983, 1984). This assumption was supported by recent findings in rats that μ receptors mediated galanin-induced feeding whereas feeding induced by fasting was dependent on a pathway mediated by κ receptors (Barton et al., 1996). Hence, endogenous opioid peptides play important roles in feeding behavior (Morley, 1995).

NPY and Food Intake

NPY, which contains 36 amino acids, is a member of the pancreatic polypeptide family found both in the peripheral sympathetic neurons and in the brain. Exogenous

NPY causes increased food intake when administered into the cerebral ventricular (Clark et al., 1984; Levine and Morley, 1984) or directly into the paraventricular nucleus in rats and mice (Stanley et al., 1984). Chronic administration of NPY into the paraventricular nucleus resulted in obesity in rats (Stanley et al., 1986). Feed intake was reduced in rats when antisense oligonucleotides which inhibit the synthesis of NYP were administrated into the paraventicular nucleus (Akabayashi et al., 1994). Also in rats, similar results have been observed by the immunoneutralization of NPY (Stanley et al., 1992; Shibasaki et al., 1993). Repeated administrations of NPY into the paraventricular nucleus caused a persistent increase in food intake and considerably accelerated the body weight gain in rats (Morley, 1987; Stanley et al., 1986; Leibowitz, 1990). These results, along with the findings that NPY decreases energy expenditure (Billinton et al., 1991), led to the hypothesis that NPY acts as an anabolic central peptide which is involved in both single-meal and long-term regulation of energy balance.

Role of Insulin

In contrast to the acute, peripheral actions of insulin, which are anabolic and calorie-storing, the chronic, CNS-mediated actions of insulin are catabolic. Hatfield et al. (1974) found that injection of large dose of insulin into the ventromedial hypothalamus reduced feeding in normal and diabetic rats. Intracerebroventricular injection of large doses of insulin also decreased food intake in rats (Brief et al., 1994; Woods et al., 1979; Plata-Salaman et al., 1986). Bilateral injection of insulin antibodies into the ventromedial hypothalamus increased food intake (Strubbe et al., 1977). McGowan et al. (1992) reported that the most sensitive sites that decreased food intake from injection of small

doses of insulin included portions of the ventromedial hypothalamus nucleus, paraventricular nucleus, dorsomedial hypothalamic nucleus and arcuate nucleus. They also confirmed that injection of insulin antibodies into the ventromedial hypothalamus increased food intake and body weight. There is a general agreement that insulin is not synthesized in significant amount in the adult animal brain (Figlewicz et al., 1996). Thus, changes of brain insulin concentrations can be considered to reflect changes in plasma insulin levels. It was hypothesized that insulin is secreted from the pancreas and enters the CNS via insulin receptor-mediated transcytosis across capillary endothelial cells which comprise the blood-brain barrier (Woods et al., 1976).

Insulin receptors are located throughout the CNS, including specific hypothalamic nucleus which are thought to be important in the regulation of food intake, body weight or energy balance. Therefore, insulin effect on food intake may be due to enhancement of the effectiveness of some satiety signals and suppression of the effectiveness of some hungry signals. When injected intraventricularly with the dose of insulin which is subthreshold for suppression of body, exogenous CCK is more effective in the suppression of food intake in rats (Riedy et al., 1995) and in baboons (Figlewicz et al., 1986, 1995). Moreover, a major target of insulin action in the CNS appears to be the hypothalamic neurons that synthesize NPY. NPY synthesis, as assessed by both mRNA levels in neuronal cell bodies that originate in the arcuate nucleus and NPY peptide levels in the paraventricular nucleus, is elevated in association with negative energy balance and insulin deficiency (e.g., fasting and/or diabetes mellitus; Sipols et al., 1995). Replacement of insulin either centrally or peripherally in either of these conditions results in a

reduction in food intake and a partial or complete suppression of NPY gene expression to levels observed under insulin-replete, or free-feeding conditions (Sipols et al., 1995). Additionally, the effectiveness of bilateral paraventricular nucleus injections of NPY to stimulate feeding is blunted in rats which receive a chronic subthreshold dose of insulin through intravenous transfusion (Sipols et al., 1995). The effects of insulin on NPY neurons in the CNS appear to be specific for the hypothalamic population only, inasmuch as neurons in other parts of the CNS are unaffected (Leroy et al., 1996). Recently, the expression of the leptin gene was reported to increase after insulin administration in rats (Saladin et al., 1995) and in vitro adipose tissue of mice (Leroy et al., 1996). Therefore, insulin may be an important regulator for expression of the leptin gene (Saladin et al., 1995; Leroy et al., 1996).

Role of Leptin

The *ob* gene, long known to be the site of a mutation associated with genetic obesity in mice, was recently cloned and found to be transcribed only in adipocytes (Zhang et al., 1994). Its product, leptin, is a polypeptide with 167 amino acids that can regulate food intake in mice (Zhang et al., 1994).

Peripheral treatment of either *ob/ob* mice or wildtype mice with dietary-induced obesity with recombinant leptin reduced food intake, body weight and percentage of body fat (Campfield et al., 1995; Halaas et al., 1995; Pelleymount et al., 1995). Intravenous injection of recombinant leptin decreased food intake in fasted normal mice (Rentsch et al., 1995). These effects appeared to be sustained for as long as leptin was administered (Pelleymount et al., 1995). In the studies of leptin's effect on energy balance, Halaas et al.

(1995) and Pelleymount et al. (1995) reported that intraperitoneal infusion of leptin normalized the reduced energy expenditure characteristic of *ob/ob* mice. All these findings suggest that the leptin plays an important role in the regulation of food intake and is necessary for a normal metabolic profile and for maintenance of normal adiposity in rodents.

Moreover, numerous studies have been conducted to elucidate the relationships of leptin with other hormones involved in appetite regulation or/and energy balance (Stephens et al., 1995; Saladin et al., 1995; Grunfeld et al., 1996). Stephens et al. (1995) reported that leptin receptors were detected in the hypothalamus and infusion of leptin into the hypothalamic nucleus decreased hypothalamic levels of NPY in rats. This indicates that leptin produced by replete adipose tissue regulates food intake and the mechanism is due to the inhibition of NPY synthesis and release. Saladin et al. (1995) reported that injection of insulin in fasted rats increased leptin mRNA to the levels of the fed controls. So, insulin may regulate leptin gene expression directly in rats independent of its glucose-lowering effects, and the increased leptin gene expression after food ingestion in rats may act through a direct action of insulin on the adipocyte (Saladin et al., 1995). In Chinese hamsters, expression of leptin was induced by endotoxin (LPS) and cytokines (TNF, IL-1) which are known to regulate adipose tissue metabolism and to induce secretion of several hormones involved in appetite regulation (e.g. CCK, glucagon and insulin, etc.) (Grunfeld et al., 1996).

Cholecystokinin and Its Effect on Satiety

The hypothesis that CCK functions as a satiety signal was based on the finding that systemic injection of CCK reduced food intake in rats (Gibbs et al., 1973). Since then, evidence from studies of both agonists and antagonists of CCK have supported this hypothesis in many species (Morley, 1985, 1995). It remains to be determined, however, where and how endogenous CCK might act to produce satiety. One popular hypothesis is that CCK acts indirectly through control of gastric emptying (Reidelberger, 1994; Figure 1). This hypothesis proposes that chyme entering the small intestine during and after ingestion of a meal stimulates secretion of CCK from endocrine cells in the upper intestine. Circulating CCK slows gastric emptying, which produces an increased rate of gastric distention, activation of vagal afferent neurons, and inhibition of the brain feeding system.

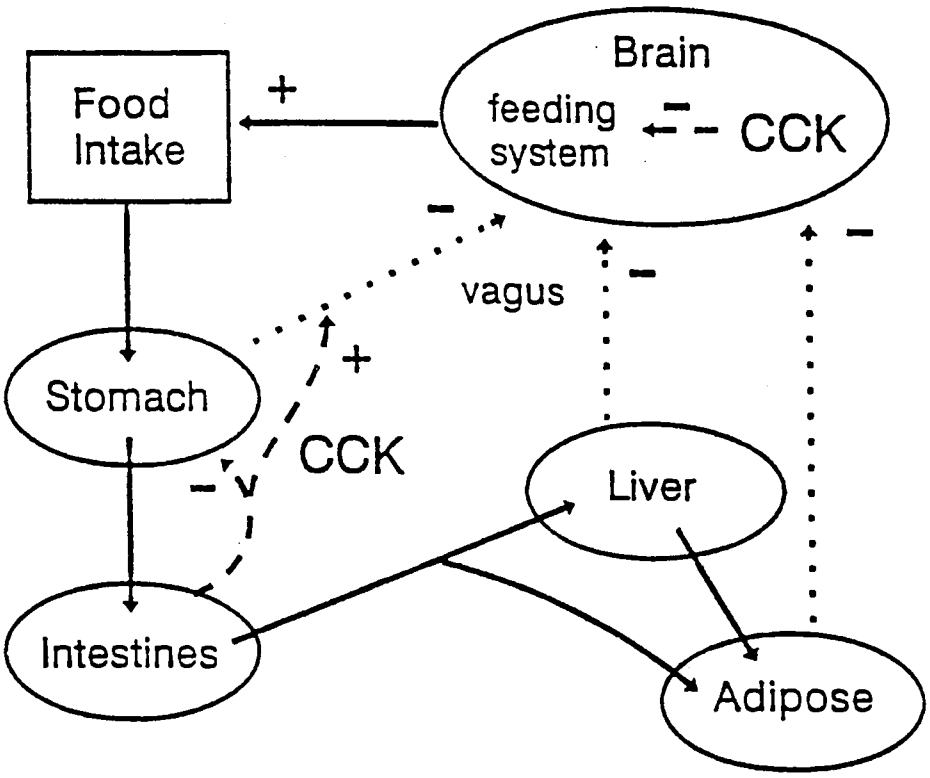

Figure 1. Schematic diagram of putative cholecystokinin (CCK) satiety mechanisms based primarily on studies of the effects of exogenous CCK on food intake. Solid lines represent putative regulatory feedback mechanisms to suppress feeding. (From Reidelberger, 1994)

It is generally agreed that heterogeneous forms of CCK exist in plasma and tissue. Among these molecular forms, the eight amino acid form of CCK (CCK-8) is thought to be the smallest bioactive (Jensen, et al., 1982) and the most biologically potent form of CCK causing satiety (Crawley et al., 1994; Reidelberger, 1994). Exogenous CCK-8 inhibits feed intake in a variety of species (sheep, Della-Fera et al., 1979; rats, Joyner et al., 1993; goldfish, Himick et al., 1994; baboons, Figlewicz et al., 1992; chickens, Covasa et al., 1994; humans, Rehfeld et al., 1978) including pigs (Anika et al., 1981; Baldwin et al., 1992). Specific antagonists for CCK-A receptors (CCKAR) (Ebenezer et al., 1990, Baldwin et al., 1992), and specific immunoneutralization of circulating CCK-8 (Pekas et al., 1993), has been shown to increase feed intake in pigs. Conversely, CCK agonists decrease feed intake in pigs (Parrott, 1993). Thus, CCK is identified as a satiety hormone in pigs.

II. Biological Characteristics of CCK Peptides and Receptors

Discovery of CCK peptides

Ivy and Oldberg (1928) reported that instillation of fat into the dog small intestine stimulated the release of a substance that activated gallbladder contraction. The substance was termed cholecystokinin (CCK). Harper and Raper (1934) discovered a hormone that was released from the porcine duodenal mucosa and stimulated pancreatic enzyme secretion; they named the hormone pancreozymin (PZ) for that reason. Mutt and Jorpes (1971) isolated a 33-amino-acid polypeptide from extracts of small intestine which exhibited the properties ascribed to CCK as well as PZ (Jorpes et al., 1966). What

originally was regarded as two distinct hormones was a single hormone. Since the action on the gallbladder was first discovered, the acronym CCK is now used instead of PZ or CCK-PZ.

CCK synthesis pathway

CCK was originally purified from porcine intestine (Jorpes et al., 1966) and sequenced as a 33 amino acid peptide (Mutt and Jorpes, 1968, 1971). Since then, molecular forms ranging in size from 5 to 58 amino acids have been identified in extracts of intestine, brain, and blood of several species. The 33-amino acid sequence and its 8-amino acid C-terminal have been identified in pigs, rats, chickens, chinchillas, dogs and humans (Eysselein et al., 1984; Fan et al., 1987; Maton et al., 1982; Rehfeld, 1978). A 39- amino acid sequence was reported in pigs (Mutt, 1980), dogs (Eysselein et al., 1984) and guinea pigs (Dockary et al., 1981). A 58-amino acid sequence was reported in cats, dogs and humans (Eberlein et al., 1988). A 47-amino acid sequence was found in frogs and in turtles (Johnson et al., 1992). As for forms shorter than CCK-33, CCK-25, CCK-18, CCK-8, CCK-7, and CCK-5 were isolated from dog intestine (Reeve et al., 1986), and CCK-22 and CCK-8 were identified from rat (Eng et al., 1984) and guinea pig intestines (Zhou et al., 1985). All of these molecular forms contain the same five amino acid sequence at the C-terminus which is identical to that of another gut hormone, gastrin. CCK-like bioactivity is conferred by sulfation of tyrosine at position 7 from the carboxyl terminus and all sulfated molecular forms larger than CCK-7 have full biological activity. The C-terminal sulfated octapeptide sequence, Asp-Tyr(SO_3H)-Met-Gly-Try-Met-Asp-Phe-NH_2, known as CCK-8, is relatively conserved across species, and appears to be the

minimum sequence for biological activity and the most biologically potent form of CCK causing satiety (Crawley et al., 1994; Reidelberger, 1994).

All molecular forms of CCK are produced by the posttranslational modifications of a single gene (Crawley et al., 1994). The gene is about 7 kilobase in size and is composed of three exons. The first exon is small and encodes only the 5' untranslated portion of the CCK mRNA. The second exon contains sequences encoding the signal peptide and prohormone regions of the peptide, and the third exon encodes the biologically important region of the hormone. Transcription of this gene in rats and in humans produces a mRNA of approximately 750 bases of which 345 encode the prepro-CCK (Deschenes et al., 1984; Takahishi et al., 1985). Prepro-CCK consists of 114 amino acids in pigs (Gubler et al., 1984) and 115 amino acids in humans (Takahishi et al., 1985) and rats (Deschenes et al., 1984). Structure of human prepro-CCK is shown in Figure 2. Posttranslational modifications of prepro-CCK include sulfation of the tyrosine, cleavage of the C-terminal Gly-Arg-Arg extension, amidation of the C-terminal phenylalanine, cleavage of the N-terminal leader sequence, cleavage of the carboxyl side of Arg-74 (Blanke et al., 1993), and cleavage of CCK-58 and smaller peptides (Turkelson et al., 1990). The various molecular forms of CCK are generated by trypsin-like cleavage (Cantor, 1989). The proposed processing pathway of human prepro-CCK has been reported (Eberlein et al., 1992) and is shown in Figure 3. Both the C-terminal sulfation and amidation are important for the biological activity (Crawley et al., 1994; Cantor, 1989). Sulfated CCK is 100 to 300 fold more potent than nonsulfated CCK (Vinayek et al., 1987), and nonamidated forms have no bioactivity (Morley et al., 1965).

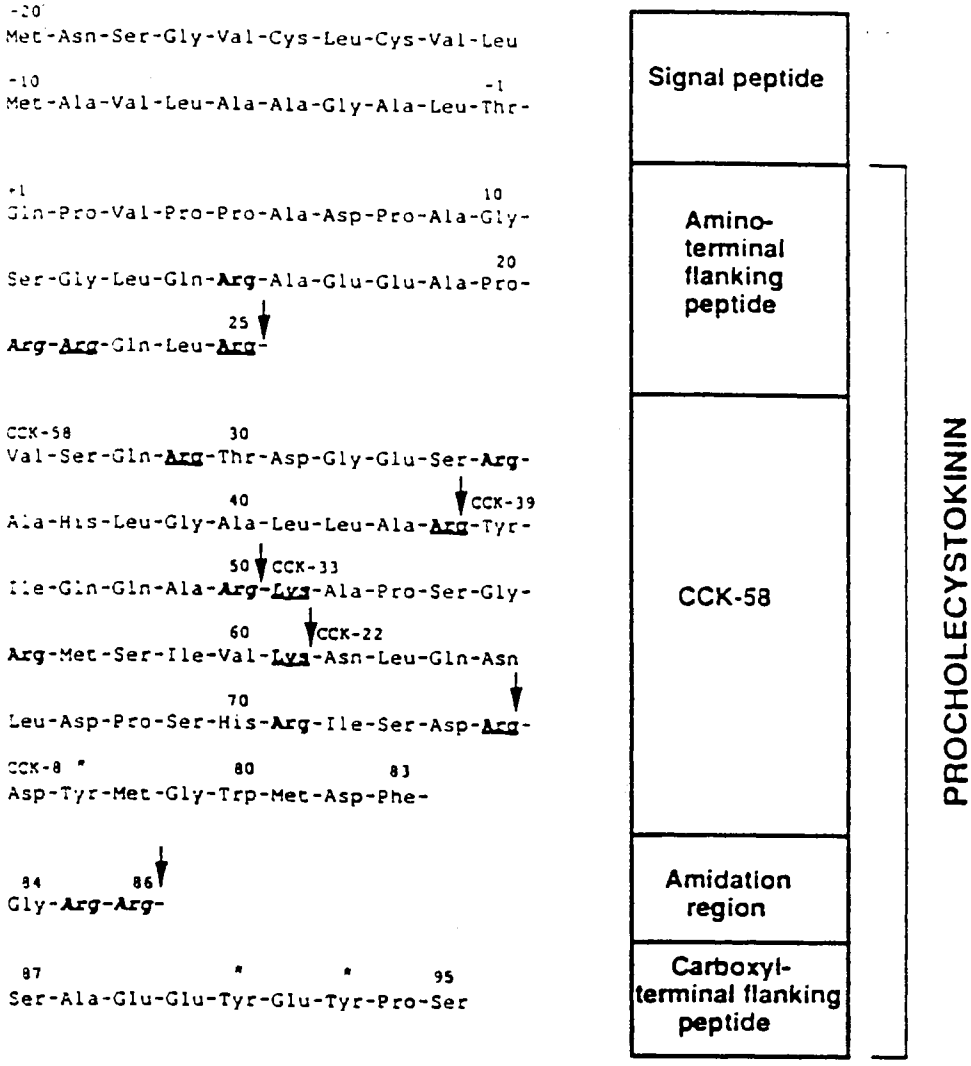

Figure 2. **Structure of human preprocholecystokinin.** The structure is shown of human preprocholecystokinin determined by cDNA studies. Basic residues are *boldface*; double basic residues are *italic*; and predicted single basic residue processing sites are *underlined.* Actual processing sites as determined by peptide purification and characterization are shown by *arrows*. Sulfation sites are shown with *asterisks*. (From Eberlein et al., 1992)

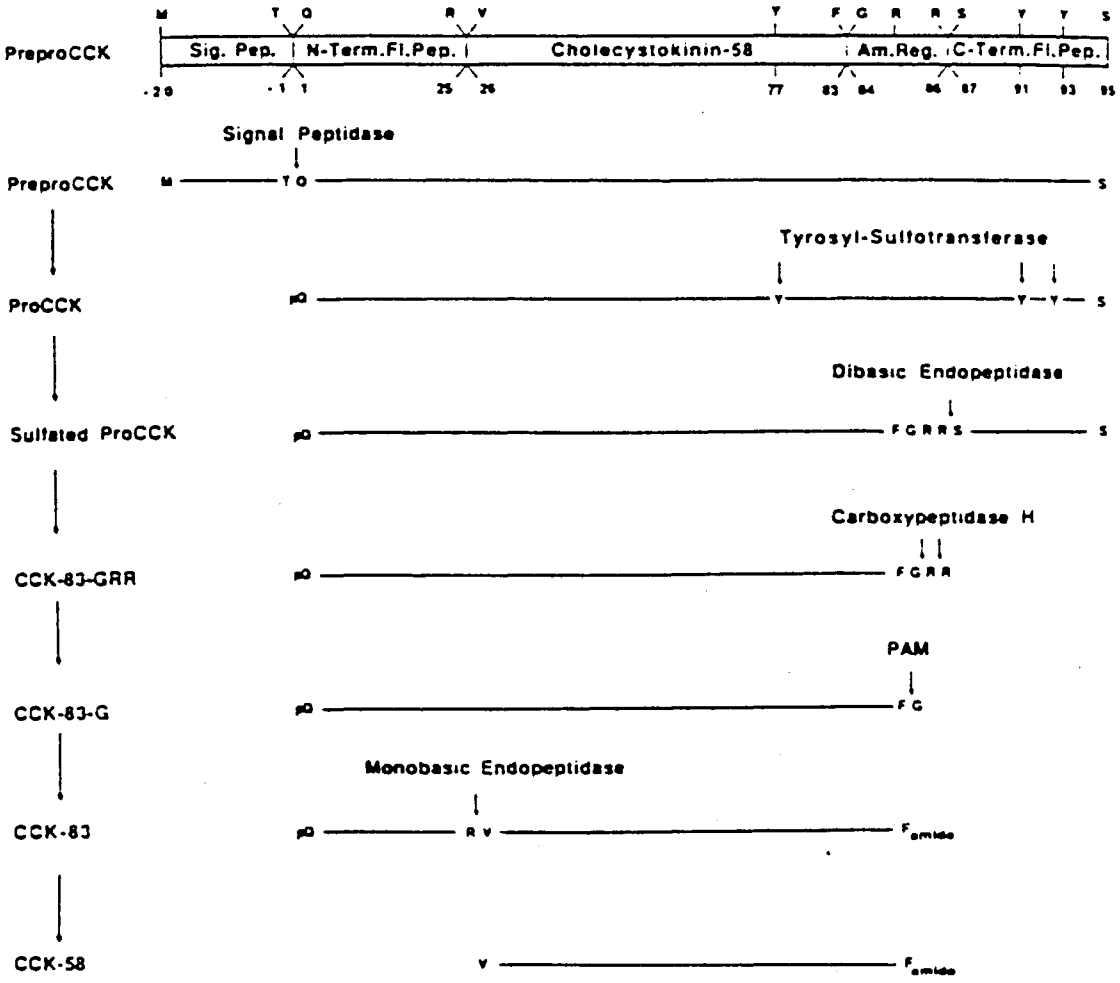

Figure 3. **Proposed processing pathway of human preprocholecystokinin.** Shown at the top are five regions of the primary preprocholecystokinin mRNA product of 115 residues. The signal peptide (*Sig. Pep.*) is 20 amino acids long. The amino-terminal flanking peptide (*N-Term. Fl. Pep.*) connects the signal peptide to CCK-58. The amidation region (*Am.Reg.*) is a tripeptide (Gly-Arg-Arg), and the carboxyl-terminal flanking peptide (*C-Term.Fl.Pep.*) is a nonapeptide. Post-translational processing is regulated by enzymes shown above the arrows. PAM, peptidylglycine α-amidating monooxygenase. Pyrrolidinecarboxylic acid (*pQ*) is formed by cyclization of the glutamyl at position 1 after the action of signal peptidase. (From Eberlein et al., 1992)

Enzymes involved in posttranslational processing include a 34,000 dalton molecular weight intestinal enzyme which degrades CCK-33 to CCK-12 and CCK-8 (Turkelson et al., 1990). In plasma, CCK-33 is cleaved to CCK-8 by enterokinase and trypsin (Gaisano et al., 1984; Mutt et al., 1981; Straus et al., 1978), and CCK-9 and CCK-10 are cleaved to CCK-8 by a trypsin-like enzyme(Deschodt-Lanckman, 1982). Sulfated CCK-8 may be cleaved into inactive fragments by aminopeptidase (Deschodt-Lanckman, 1982).

In the study of CCK peptides in human and rat plasma, half-lives of sulfated CCK-8 were 50 and 17 min, respectively, and that of desulfated CCK-8 were shorter, at 18 min in human and 5 min in rats (Koulisher et al., 1982).

Localization of CCK production

1. Distribution of CCK in CNS

Radioimmunoassay (RIA), immunohistochemistry and in situ hybridization have been used to study the distribution of CCK peptides in the CNS. Neurons containing CCK are widely distributed throughout the brain except the cerebellum (Beinfeld et al., 1981). CCK or CCK mRNA is present in a very high concentration in the cerebral cortex (Crawley et al., 1994). The distribution of immunoreactive CCK in brain regions of the rat was reported by Beinfeld et al. (1981; Table 1). Most of the information regarding the localization of CCK in the brain has been obtained from studies of the rat, and similar distribution patterns of CCK were also observed in other species such as pig (Rehfeld, 1978); guinea pig (Larsson et al., 1979); human (Beinfeld et al., 1981; Emson et al., 1982; Geola et al., 1981); cattle (Braden et al., 1981), rhesus monkey (Beinfeld et al., 1983) and sheep (Dockray et al., 1978).

Table 1. Immunoreactive CCK in Brain regions of the rat [a]

Region	ng CCK-8 equivalents/ g net weight	ng CCK-8 equivalents/ brain region
Cortex	528 ± 129	414 ± 75
Striatum	328 ± 73	37 ± 13
Hippocampus	274 ± 0.3	330 ± 7
Septum	237 ± 68	4 ± 0.8
Anterior hypothalamus	232 ± 58	2 ± 0.3
Olfactory bulb	204 ± 61	10 ± 2
Thalamus	191 ± 36	18 ± 2
Amygdala	164 ± 57	2 ± 0.2
Mesencephalon	159 ± 21	25 ± 0.6
Posterior hypothalamus	157 ± 6	2 ± 0.5
Pons	28 ± 3	3 ± 0.4
Medulla	25 ± 6	3 ± 0.6
Pituitary gland (whole)	25 ± 6	0.3 ± 0.4
Cerebellum	1 ± 0.3	0.3 ± 0.5

[a] Means ± SEM, n = 3. Striatum included equdate putamen, globus pallidus, and nucleus accumbens. Tissues were removed, frozen and weight before extraction (From Reinfeld et al, 1981).

Rehfeld (1978) used RIA in combination with high performance liquid chromatography (HPLC) to show that heterogeneous CCK was present in the brain as well as in the small intestine of both humans and pigs. To date, CCK-8 has been isolated from the brains of a substantial number of mammalian species including, rabbits (Straus et al., 1977), pigs (Rehfeld, 1978), sheep (Dockray et al., 1978), and humans (Miller et al., 1984). CCK-58 has also been isolated from dog (Eyssenlein et al., 1984) and pig brain (Tatemoto et al., 1984). However, neither CCK-33 nor CCK-39 has been isolated

from the brain of any species. How the posttranslational processing occurs in the brain is still unknown. There may be different cleavage enzymes in the brain.

2. Peripheral system

a) Gastrointestinal tract

High concentrations of CCK are present in the mucosa of the duodenum and jejunum in humans, dogs and rats (Soll et al., 1985). CCK-33 or larger forms are found in high concentrations in the mucosa in the duodenum and jejunum (Greeley et al., 1984a). Use of a C-terminal antiserum for CCK-8, Greeley et al. (1984a) determined that concentrations of CCK-33 and larger forms were 5 to 20 times higher than CCK-8 in the mucosa of the duodenum and jejunum. CCK-8 has also been detected in the gastric atrium, but not in liver, kidney, spleen, or pancreas of the rat, rabbit or dog (Brand et al., 1981).

b) Nervous system

In the aforementioned studies, extracts of the whole tissues were used. Investigators have also found CCK in neural and nonendocrine cells of the gastrointestinal tract (Larsson et al., 1979). CCK is expressed in a small but significant population of neurons in both the myenteric and submucous plexus of the enteric nervous system (Schultzberg et al., 1980; Furness et al., 1985). In addition, CCK fibers were identified in the mucosa of duodenum and jejunum (Larsson et al., 1979). CCK was also found in nerve terminals of pancreatic islets (Rehfeld et al., 1980) and in vagal afferent fibers in dogs and cats (Dockray et al., 1981; Rehfeld et al., 1983; Zarbin et al., 1981).

c) Plasma

CCK exists in heterogeneous forms in plasma and tissue (Crawley et al., 1994). However, the exact molecular nature of CCK in plasma has been much debated because of the presence of interfering factors (e.g., gastrin) and differences in the specificity of the assays employed. Investigators have pretreated plasma in various ways including extraction with acid or ethanol as well as application of resins to decrease the interference (Jansen et al., 1983; Go et al., 1980; Liddle et al., 1985; Walson et al., 1982; Cantor and Rehfeld, 1987). Although these methods remove interfering compounds, none resulted in complete recovery of added CCK. Various values in basal levels of CCK have been reported because of the use of antibodies with different specificities, different methods of extraction, and different methods and forms of CCK used for iodination (Jansen et al., 1983; Go et al., 1980; Liddle et al., 1985; Walson et al., 1982; Cantor and Rehfeld, 1987; Chang et al., 1983; Kothary et al., 1983; Maton et al., 1982).

With the use of antibodies specific for CCK-33 and CCK-39, values for CCK-33 and CCK-39 in unextracted plasma from humans, dogs and pigs were in the range of 16 to 50 pmol/L (Chang et al., 1983; Jansen et al., 1983; Kothary et al., 1983; Maton et al., 1982). With the use of similar antibodies, the range of values in plasma pretreated by ethanol extraction was 1-4 pmol/liter (Lilja et al., 1982; Lonovics et al., 1981; Gaisano et al., 1984).

In extracted plasma, the use of antibodies against both CCK-8 and CCK-33 or CCK-8 alone, resulted in measurement of fasting plasma levels of 1 to 8 pmol/L in rats (Maton et al., 1982; Eysselein et al., 1984; Chang et al., 1983; Calam et al., 1982; Greeley et al., 1984b). In humans, the basal concentration was 1 to 3 pmol/L (Rehfeld,

1978; Cantor, 1986; Jensen et al., 1983; Liddle et al., 1985; Dockray et al., 1978), and similar concentrations have been reported in rats (Liddle et al., 1984) and pigs (Cantor and Rehfeld, 1989).

Reports from different laboratories vary as to the exact molecular patterns of the heterogeneity of CCK in plasma. Kothary et al. (1983) reported that human plasma contained 10% of CCK peptides larger than CCK-39, 3% of CCK-33 and CCK-39, 11% of CCK peptides with molecular weight between CCK-8 and CCK-33, 58% of CCK-8 and 18% of CCK-4. Liddle et al. (1985) reported that predominant CCK-33/39, lesser amounts of CCK-22 and CCK-8 and no CCK-58 were in postprandial plasma. Cantor and Rehfeld (1987) found a predominance of CCK-33/39 and CCK-8, and moderate to small amount of CCK-22 and CCK-58 forms, respectively. Jansen and Lamas (1983) reported that 8 to 40% of CCK-58, 44 to 60% of CCK-33 and CCK-39, 15 to 27% of CCK peptides greater than CCK-14 but less than CCK-33 were present in human plasma, but no CCK-8 was detected. Calam et al. (1982), however, reported CCK-8 to be the predominant form and a lesser amount of CCK-33 in human plasma. The molecular distribution of CCK in human and pig plasma is almost identical (Liddle et al., 1985; Cantor and Rehfeld, 1989), but CCK-22 and CCK-8 have been the only forms detected in rat plasma (Liddle et al., 1984). CCK-58 was reported to be the major form of CCK in dogs (Eysselein et al., 1987; Sun et al., 1992). These data indicate that various patterns of CCK occur in different species.

CCK receptors---Characterization, distribution and function

There are two subtypes of CCK receptors. They are pharmacologically classified on the basis of their affinity for the peptide agonists CCK and gastrin, which share the same C-terminal pentapeptide amide sequence but differ in sulfation at the sixth (gastrin) and seventh (CCK) tyrosyl residues, and by recently developed subtype-specific antagonists (Presti et al., 1993). CCK A-receptor (CCKAR) was first characterized on rat pancreatic acinar cells (Sankaren et al., 1980), and the second subtype with a different pharmacology was discovered in the brain and termed as CCK B-receptor (CCKBR; Innis and Synder, 1980). CCKAR is highly selective for sulfated analogues of CCK and the antagonist L-364,718 (Lotti et al., 1989), whereas CCKBR has a similar affinity for both sulfated and nonsulfated peptide analogues of CCK/gastrin peptides (Saito et al., 1981)

Table 2. Characteristics of the two subtypes of CCK receptors [b]

Nomenclature	CCKAR	CCKBR
Potency order	CCK-8 >> gastrin, des-CCK-8CCK- (~500 to 1000 fold) CCK-8 >>> CCK-4 (~10,000 fold)	8 = gastrin, des-CCK-8 (0 to 10 fold) CCK-8 > CCK-4 (10 to 600 fold)
Selective antagonist	L-364,718	L-365,260
G protein coupled	Yes	Yes
Signal transduction		
PLC, IP, DAG	Yes	Yes
cAMP	Yes	No

[b] CCK, cholecystokinin; CCK-8 and CCK-4, cholecystokinin octapeptide and tetrapeptide, respectively; CCKAR and CCKBR, CCK-A and CCK-B receptors, respectively. G Protein, guanine nucleotide-binding regulatory protein; PLC, phospholipase C; IP, inosito phosphate; DAG, diacyglycerol. (From Wank, 1995)

Table 3. Location and function of CCK receptor subtypes [c]

CCKAR	CCKBR
Pancreatic acini	Throughout CNS
Enzyme secretion ↑; growth.	Anxiety, panic attack; Dopamine release ↓.
Gastric mucosa	Gastric mucosa
Chief cell---pepsinogen ↑;	Growth;
D cell---SMS ↑;	Parietal cell---acid ↑;
Gallbladder and GI smooth muscle	ECL cell---histamine ↑;
contraction; motility.	Chief cell---pepsinogen ↑;
Select area of CNS and PNS	Immune cells
Satiety;	T lymphocytes; monocytes. ? function.
Dopamine release ↑;	
Opioid anlgesia;	
Neoplastic cells---growth	Neoplastic cells---growth
AR42J	ARJ42; Leiomysarcoma; SCLC;
CHP212	Gastric carcinoma; Colonic carcinoma.

[c] CCK receptors are classified into a major subtypes on the basis of their pharmacology. Arrows indicate the change in function mediated by each receptor subtype. SMS, somatostatin analogue. CNS and PNS, central and peripheral nervous system, respectively; GI, gastrointestinal; ECL, enterochromaffine-like. SCLC, small cell lung carcinoma (From Wank, 1995)

and the antagonist L-365,260 (Lotti et al., 1989). Molecular genetics analysis indicates that the CCKBR and the gastrin receptor are derived from the same gene and are the same protein (Wank et al., 1992). Characteristics of the CCKAR and CCKBR, and their locations and functions are summarized in Table 2 and Table 3, respectively.

Using radioligand and autoradiography, high levels of CCKAR binding in peripheral organs, and high levels of CCKBR binding in the CNS have been detected (Wank, 1995). Stimulation of CCKAR with physiological concentrations of CCK causes pancreatic exocrine enzyme secretion (Sankaran et al., 1980), endocrine islet cell

secretion of insulin (i.e., the incretin effect of CCK; Karlsson et al., 1992), secretion of pancreatic polypeptide (Liddle et al., 1990), and pancreatic growth (Zucker et al., 1989). CCKAR in the stomach mediate secretion of pepsin from gastric chief cells (Gaisano et al., 1984) and release of somatostatin from D cells of gastric mucosa resulting in the inhibition of acid secretion. Also, CCKAR on smooth muscle cells have been reported to cause gallbladder emptying (Bitar et al., 1982).

CCKAR are located predominantly in the peripheral, however, they are also present in nerve fibers in select areas of the CNS and peripheral nervous system (PNS) (e.g. vagus nerve) where they mediate the satiety effect of CCK released from the small intestine or enteric neurons after a meal (Corp et al., 1993; Ritter et al., 1994; Smith et al., 1981, 1985; South et al., 1988). CCKAR in the medial posterior nucleus accumbens regulate dopamine release (Grawley, 1991) and those in the dorsal horn of the spinal cord in primates antagonize opioid analgesia tolerance (Baber et al., 1989). CCKAR on the anterior pituitary are suggested to mediate the release of adrenocorticotrophic hormone and β-Endorphin (Kamilaris et al., 1992). CCKAR are also found in several cell lines, e.g., the rat pancreatic carcinoma cell line (AR42J) (Logsdon, 1986) and the human neuroblastoma cell line (CHP212) (Klueppelberg et al., 1990), leading to the conclusion that they may mediate cell growth.

CCKBR are predominantly found throughout the CNS. CCK acting on CCKBR in the anterior nucleus accumbens, unlike CCKAR in the posterior nucleus accumbens, has an inhibitory effect on dopamine release (Crawley, 1991). This suggests a possible role in the pathogenesis of dopaminergic related movement and behavioral disorders in

the human (Crawley, 1991). CCKBR are also found to mediate the regulation of anxiety by peripheral and central CCK (Harro et al., 1993). CCKBR are present on immune cells such as monocytes (Sacerdote et al., 1991) and T lymphocytes (Lignon et al., 1991), where their functions are still unknown. Like CCKAR, CCKBR are also detected on tumors and tumor-derived cell lines such as the AR42J (Logsdon, 1986) and human leiomyosarcoma cells (Pearson et al., 1989) where they may stimulate growth.

III. Animal Models in The Study of Genetic Variation in Appetite

Food intake is a quantitative trait affected by many genes. In normal animals, each of these genes has relatively small effect on phenotype. Genetic variation in appetite may be due to the simultaneous segregation of many genes involved in the control of food intake and the regulation of energy balance. Genetically-inherited models of obesity in rodents have been clearly established to study the genetic nature of the regulation of food intake. The identification of the genes involved in the development of obesity in these animal models may lead to the elucidation of the underlying basis for quantitative genetic variation in appetite and energy balance.

Yellow obese ($A^y a$) mouse

Obesity in the $A^y a$ model is inherited through a dominant gene (the a*gouti* gene) (Bray et al., 1979). Recent studies have determined that the *agouti* gene is located on chromosome 2 in the mouse and on chromosome 20 in the human (Kwon et al., 1994). The mouse *agouti* gene was cloned and found to encode a 131 amino-acid protein

(Bultman et al., 1991, 1992; Miller et al., 1993; Michaud et al., 1994; Lu et al., 1994). The *agouti* protein antagonizes the binding of α-melanocyte-stimulating hormone (α-MSH) and certain other pro-opio-melanocortin peptides (POMC) to their receptors (Lu et al., 1994; Willard et al., 1995). One of the effects of α-MSH is to stimulate food intake (Morley, 1987; Shimizu et al., 1989).

Obesity is associated with a change of pigmentation from black to yellow because the genes controlling obesity and the agouti coat colors are so closely linked. To date, 34 or more alleles at the mouse *agouti* locus have been identified (Lyon et al., 1990; Siracusa, 1994). These alleles can be arranged in a phenotypic dominance hierarchy (Lyon et al., 1990). A^{vy} is one of the four dominant agouti mutations associated with pleiotropic effects (the other three are A^{y}, A^{iapy} and A^{iy}). The most prominent effects are obesity, hyperinsulinemia and hyperphagia (Yen et al., 1994). Yellow A^{vy}/- mice eat 10 to 36% more than their non-A^{vy} siblings, depending on genetic strain background (Yen et al., 1994). The pituitary of yellow A^{vy}/a mice has a reduced α-MSH : desacetyl-α-MSH ratio (Bray et al., 1988). Because desacetylated α-MSH is more potent than α-MSH in stimulating food intake (Shimizu et al., 1989), reduced acetylation of α-MSH may play a role in A^{vy}-associated hyperphagia.

Obese (*ob/ob*) mouse

Obesity in *ob/ob* mice is caused by an autosomal recessive mutation (Ingalle et al., 1950). The *ob/ob* mice are obese, hyperphagic, hyperglycemic, hyperinsulinemic, insulin resistant and hypoactive (Bray et al., 1979). CCK content in brains of *ob/ob* mice

is lower than their lean *(-/ob)* littermates (Straus and Yalow, 1979). Furthermore, *ob/ob* mice overexpress the neuropeptide Y gene in hypothalamus (Wilding et al., 1993).

To date, the *ob (leptin)* gene has been located on chromosome 6 in mice (Zhang et al., 1994), on chromosome 7 in human (Green et al., 1995), on chromosome 4 in bovine (Stone et al., 1996) and on chromosome 18 in porcine (Sasaki, et al., 1996; Neuenschwander et al., 1996). As described in the previous section, studies have revealed that leptin plays an important role in the regulation of food intake and energy balance in rodents (Halaas et al., 1995; Pelleymount et al., 1995; Rentsch et al., 1995). However, to my knowledge, the function of leptin has not been clarified in other species.

Diabetic (*db/db*) mouse

The recessive mutation of the *db* gene originally arose in the C57BL/KsJ strain of mice at the Jackson Laboratories (Coleman, 1978). The *db/db* mouse is distinguished from other obese mouse models by the appearance of ketosis. Like all other obese rodent models, the *db/db* mouse is hyperphagic, hyperinsulinemic and obese (Bray et al., 1979). Increased levels of leptin mRNA were reported in adipose tissue of the *db/db* mouse (Trayhum, 1996). Also, leptin suppressed food intake and decreased body weight dramatically when administered to normal and *ob/ob* mice, but not *db/db* (diabetic) mice (Stephens et al., 1995). Thus, the *db/db* phenotype seems to reflect a defect in the leptin action. Recent studies have determined that the *db* gene encodes the leptin receptor and is located on chromosome 4 in the mouse (Tartaglia et al., 1995; Lee et al., 1996).

Fatty *(fa/fa)* rat

The autosomal recessive mutation (*fa*) in the rat was first described by Zucker and Zucker (1961) and arose from a cross between Sherman and Merck stock M rats. Homozygous recessive (*fa/fa*) rats are obese, hyperphagic, hyperinsulinemic and hyperlipemic. Zucker obese rats (*fa/fa*) are less sensitive to the satiety effects of CCK (Moos et al., 1982) and have elevated NPY production in the arcuate nucleus (Beck et al., 1990; Sanacora et al., 1990). In addition, the level of leptin mRNA in adipose tissue is higher in *fa/fa* rats (Trayhum, 1996). The *fa* locus maps to chromosome 5 in the rat (Chua et al., 1996; Iida et al., 1996; Murakami and Shima, 1995) and the normal allele (*FA*) encodes the leptin receptor (Chua et al., 1996; Chen et al., 1996).

OLETF rat

Results from a Japanese laboratory described physiological and molecular studies performed on an inbred strain of rats, Otsuka Long-Evans Tokushima Fatty (OLETF) rats. The OLETF rat was developed in 1992 as a model of human-non-insulin-dependent diabetic mellitus from an outbred colony of spontaneously diabetic rats (Kawano et al., 1992). Male rats develop late-onset hyperglycemia (after 18 wk of age), mild obesity, and insulin deficiency (after 65 wk of age). Elevation of plasma CCK by either exogenous CCK administration or endogenous secretion after bile-pancreatic juice diversion failed to stimulate pancreatic exocrine secretion in the OLETF rat (Funakoshi et al., 1995). Cerebroventricular administration of sulfated CCK-8 did not inhibit daily food intake in the OLETF rat (Miyasaka et al., 1994). The incretin effect of CCK, a function mediated by CCKAR on islet cells, is also absent in the OLETF rat (Funakoshi et al., 1995) and no CCKAR mRNA is detected in either pancreas or hypothalamus (Miyasaka et al., 1994;

Funakoshi et al., 1994, 1995). These physiological and molecular studies suggest that the OLETF rat may represent a naturally occurring CCKAR gene "knockout" rat and can be used as a model to study the physiological function of CCKAR, especially for feeding behavior (Wank, 1995).

IV. Animal Population in the present Study

1) Establishment of divergent selection lines

A study was started in 1979 to investigate direct and correlated responses to divergent selection for postweaning average daily gain (ADG). In the first year, Hampshire boars were purchased in pairs from central test stations in Iowa, Missouri, Nebraska and Oklahoma. Boars were evaluated on the index recommended by the National Swine Improvement Federation that emphasized increased ADG, decreased backfat, and improved feed efficiency as described by Woltmann et al. (1992). In each pair, one boar had a high index value (index value $\geq$ 118) and the other a low index value (index value $<$ 90). These boars were mated to a population of Duroc x Yorkshire x Landrace x Spotted crossbred gilts from a previous study. In the second year, pairs of Duroc boars were purchased on the same index criteria as in the first year. Duroc boars were mated with females produced in the first year. The fast growth line (F) was generated from pigs sired by high-indexing Duroc boars and out of gilts sired by high-indexing Hampshire boars. The slow growth line (S) was derived from pigs sired by low-indexing Duroc boars and out of gilts sired by low-indexing Hampshire boars. The lines were then closed and underwent ten generations of selection for either fast (F) or slow (S)

ADG from 9 wk of age to 100 kg. Selection lines were replicated in spring- and fall-farrowing groups. The spring replicate farrowed during mid-March through April and the fall replicate farrowed during mid-September through October. Complete replacement of boars and gilts resulted in a generation interval of 1 year.

2) Responses to selection

Response to selection in F and S through generation four was reported by Woltmann et al. (1992). Barrows and gilts from F grew 0.16 kg/day faster than those from S. Pigs from F also ate 0.41 kg/day more feed and had 0.10 cm more backfat at 105 kg than those from S. After five generations of selection, average daily feed intake for F was 23% more than for S (Woltmann et al., 1995). This divergency in feed intake and ADG continued through further selection (Clutter et al., 1995). An evaluation of F and S, reflecting seven generations of selection is summarized in Tables 4 through 6 (Clutter, 1992).

Physiological effects of the divergent selection in F and S were also investigated. Norton et al. (1989) measured several plasma characteristics in gilts sampled from F and S after generation four. Fasting concentrations of insulin and glucose in peripheral plasma were greater in F than in S gilts, but nonesterified free fatty acid levels were greater for S gilts than for F.

Characteristics of GH, IGF-I and IGF-binding proteins (IGFBP) were studied in gilts sampled from the lines after seven generations of selection (Clutter et al., 1995). Repeated blood samples were obtained from gilts (~55kg BW) during a period of feed deprivation and again during refeeding. None of the characteristics of plasma GH concentrations was significantly different between F and S gilts. Plasma IGF-I

Table 4. Average daily gain and backfat thickness of F and S Barrows and Gilts

Sex	Line	n	ADG, kg	ADJBF, cm [D]
Barrows	F	173	0.91 (0.02)[d]	3.38 (0.01)
	S	129	0.7 (0.02)	2.72 (0.01)
Gilts	F	365	0.88 (0.02)	3.15 (0.01)
	S	218	0.62 (0.02)	2.31 (0.01)

[d] Mean ± S.E.

[D] Average backfat thickness was measured by ultrasonic probe adjusted to 105 kg.

Table 5. Average daily feed intake and feed conversion ratio of F and S Barrows and Gilts [E]

Line	n	ADFI, kg	Feed : Gain
F	538	2.71 (0.04)[e]	3.10 (0.02)
S	347	2.01 (0.04)	3.14 (0.02)

[e] Mean ± S.E.

[E] ADFI was evaluated on the pen basis.

Table 6. Carcass Characteristics of F and S Barrows

Line	n	ADJBF, cm	Lean %	Lean ADG, kg
F	73	3.38 (0.02)[f]	46.3 (0.39)	0.33 (0.01)
S	86	3.10 (0.02)	49.1 (0.35)	0.28 (0.01)

[f] Mean ± S.E.

concentrations were greater in F than in S gilts during feed deprivation and during refeeding. A greater IGFBP2 and IGFBP3 activity was detected in S. Therefore, response to selection in these lines may have produced some alterations in the IGF-I pathway.

V. Genetic Improvement, Molecular Markers and Quantitative Trait Loci

Genetic improvement with the aid of molecular tools

Genetic improvement as a result of single-trait selection is determined by the heritability of the trait, the amount of variation among potential replacements, the genetic superiority of the selected replacements, and the time interval required to turn over a generation:

$$R \text{ per year} = h^2 \times S/L$$

where

R is the annual response to selection

h^2 is the heritability of a selection trait

S is the selection differential

L is the generation interval

To enhance annual genetic improvement, breeders need to either increase the heritability of a trait (or the accuracy with which the most genetically superior animals are identified), increase the selection differential (or the measured superiority of selected replacements), or decrease the generation interval.

Selection of parents is often based on a combination of phenotypic measurements, including performance of the individual itself, its ancestors, its siblings and its progeny. All of the phenotypic information is analyzed with statistical methodologies to obtain breeding values or Expected Progeny Differences (EPDs) for individuals in a population, and these EPDs information can be used to make selection decisions. However, it is a time-consuming task to obtain these phenotypic data.

Production traits like growth rate, body composition and litter size are quantitative in nature, which by definition means they are affected by many genes each of which has a relatively small effect. By selecting the animals with superior performance to be parents in the population, the genetic merit of the population is then improved by increasing the frequencies of superior gene forms and reducing the frequencies of inferior gene forms. However, little is known about the specific genes that affect the various production traits and which forms of those genes are desirable.

Now, due to developments in quantitative and molecular genetics, it is possible to study the genetic basis of quantitative traits at the DNA level. These studies include to identify, map, and measure the effects of quantitative trait loci (QTLs) or economic trait loci (ETLs). QTLs are those loci with relatively minor, quantitative effects on phenotypes of production traits, while ETLs are all loci with relatively minor, quantitative or with major effects on economically important traits. In order to determine which genes or chromosomal regions are important in the control of production traits and to identify superior forms of those genes to be used in the selection, a genetic marker map of the genome is necessary. When the map is sufficiently saturated with markers, every QTL or ETL of interest will reside next to a marker. By monitoring the inheritance of specific

marker alleles and the corresponding phenotypic performance of offspring that receive them, markers linked to desirable sequences at QTLs or ETLs may be identified.

Molecular Markers, Mapping and QTL

There are two major approaches for QTL or ETL studies, the candidate locus approach and the genome scanning approach.

In the candidate locus approach, a restriction fragment length polymorphisms (RFLPs) within a gene with known biological functions is used as a genetic marker. Genotypies at the candidate locus are are determined for individuals in a segregating resource family and association to the phenotype of interest is determined. For example, in a study of individual genes affecting murine growth, Winkelman and Hodgetts (1992) scored molecular variants at the insulin-like growth factor 2 (IGF-2) and growth hormone (GH) loci since these growth factors are known to be important in somatic growth. Several genes of major effect on quantitative traits in farm animal species have been detected in recent years. In pigs, discoveries have been made which indicate that the estrogen receptor locus (ESR) influences litter size (Rothschild et al., 1996) and pituitary transcription factor 1 (PIT1) is associated with birth weight and backfat (Yu et al., 1996). Insulin-like growth factor 1 (IGF1) locus is associated with average daily gain (ADG) (Casas-Carrillo et al., 1997). Detection of the associations of these genes with the phenotypic performance provides useful scientific tools for the investigation of the genetic causes of phenotypic variation. There are several advantages to the candidate locus approach:

1) The results are interpretable in relation to trait physiology;

2) It provides direct measures of genotypic values.

In the genome scanning approach, genotypes are measured at a large number of marker loci including candidate gene markers and anonymous markers. The anonymous markers are usually in nonfunctional sequences of DNA with unique sequence variation, such as microsatellites---DNA sequences made up of tandem repeats and alleles based on variable number of repeats (e.g. $(CA)_n$ repeats). Amplified microsatellites may be scored by agarose or polyacrylamide gel electrophoresis if alleles differ in size (Figure 4). Ideally, marker loci spaced approximately 5 to 10 centiMorgans throughout the genome are evaluated. With such a high density of marker loci, it is likely that alleles at marker loci will be in linkage disequilibrium with QTL alleles resulting in a correlation between quantitative trait values and marker genotypes. The advantages of this approach are:

1) It surveys the entire genome;

2) It allows discovery of previously unknown genes.

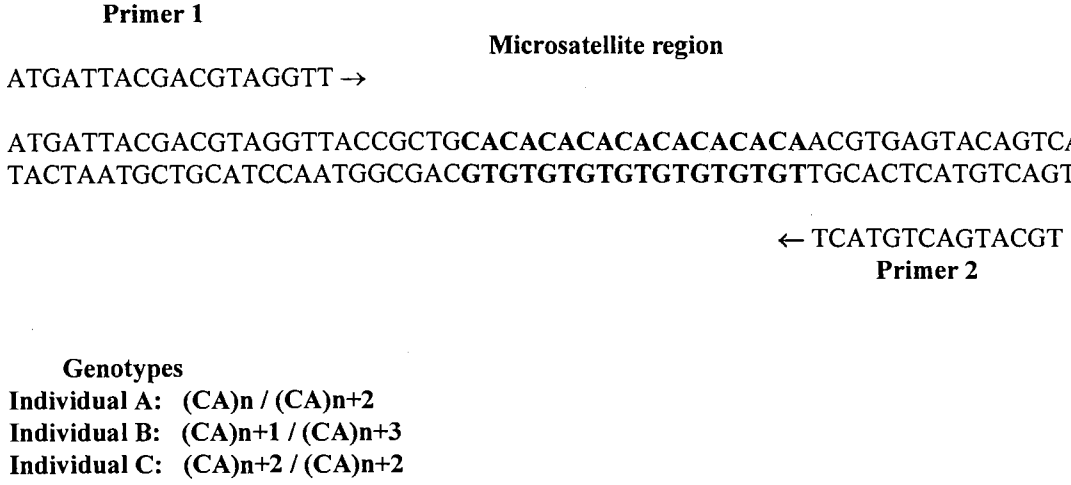

Figure 4. Principles for microsatellite amplification and scoring of alleles by gel electrophoresis.

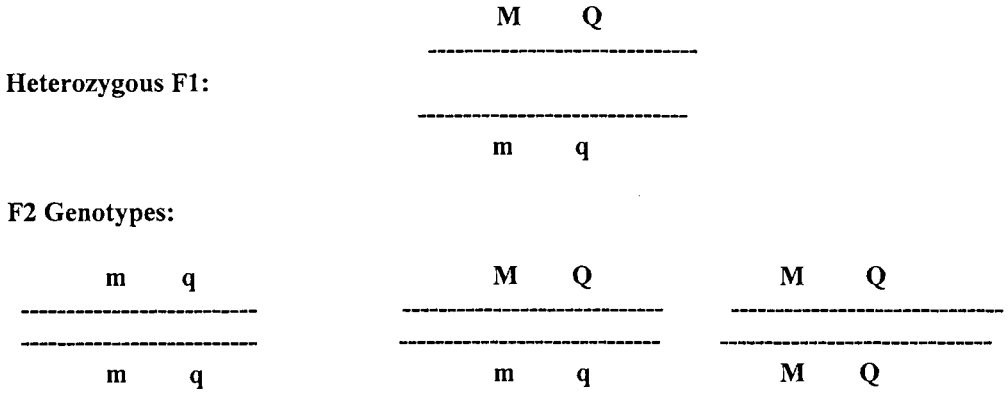

Figure 5. An example of F1 individual heterozygous for both a DNA marker (with forms M and m) and linked QTL locus of interest (with forms Q and q). Genotypes of the marker, and along with them genotypes of the linked QTL, segregate in production of the F2. Comparing performance for production traits of F2 individuals that receive the different marker genotypes determines the value of QTL linked to segregating marker. This example depicts only the pair of chromosomes on which the marker and the QTL reside and assumes they are linked tightly enough that there is no crossing over between them.

Theoretically, studies to locate QTLs or ETLs that affect important traits are straightforward. By following the inheritance of alleles of markers throughout the genome, and determining the associations of those marker alleles with trait(s) of performance, regions of the genome harboring QTLs (ETLs) can be identified. If enough evenly spaced markers are used, wherever a QTL resides it will be linked to one of the markers. The most effective design is one in which segregating marker alleles are studied in an F2 resource family produced by crossing very divergent grandparent stock (e.g. wild boar x domestic female).

A simple example involving a single marker and linked QTL is shown in Figure 5. The cross of divergent lines should result in an F1 that is heterozygous for the marker and QTL alleles. The expectation for the F2 is the 1 : 2 : 1 ratio for three possible genotypes at each locus. By comparing average performance of the marker genotype groups for traits of interest (e.g. growth rate, fat deposition, food intake), a linked QTL can be detected.

In pigs, this genomic scan approach with microsatellite markers has been used to identify chromosomal regions or "hot spots" controlling fatness (Andersson et al., 1994; Rothschild et al., 1996), meat quality (Le Roy et al., 1990), growth (Andersson et al., 1994; Casas-Carrillo et al., 1997b) and ovulation rate (Wilkie et al., 1996; Rathje et al., 1997).

Comparative Mapping

During evolution a dynamic process of chromosomal rearrangements occurs. DNA segments are inserted or deleted, chromosomes are translocated or split, and genomes are polyploidized. Related species share many features of genomic organization and chromosomal structure as seen directly from their karotypes (Nash et al., 1982; Sawyer et al., 1986; O'Brien et al., 1988; Levan et al., 1991). In comparative mapping, the genomic localization of two or more loci is compared between species (Nadeau, 1989; O'Brain et al., 1991). If the loci are linked or syntenic in more than one species, a conserved chromosomal segment is present. These conserved segments are of particular importance for gene mapping in farm animals. Whenever a locus is localized to a particular conserved chromosomal region, the map can be compared with those of human and mice which are much more dense, to find potential candidate genes. By using this comparative mapping approach, positional candidates for the RN gene for meat quality has been identified in pigs (Milan et al., 1995). OTF1 has been located on porcine chromosome 4 within a large syntenic group conserved on HSA 1 (Tuggle et al., 1995). Thus, the positional candidates for a QTL or ETL in this syntenic region detected on HSA 1 will probably be mapped to SSC 4.

Marker-Assisted Selection

In animal industries, the ultimate purpose of gene mapping is to apply the derived information in breeding programs. This can be carried out by selection on favorable QTLs via selection on linked markers, i.e. marker-assisted selection (MAS) (Soller et al., 1982, 1990; Rothschild et al., 1990). Better understanding of gene action will also aid in building models of phenotypic variation, permitting breeding values to be more accurately estimated (Weller, 1996). Although considerable genetic enhancement in some performance traits has been achieved with traditional methods (Buchanan et al., 1993), more genetic improvement may be obtained by application of MSA (Soller, 1994; Weller, 1996).

MAS has potential to increase genetic response to single trait selection in three ways: 1) increase the accuracy for predicting superior candidates; 2) increase the selection intensity by considering more young animals as potential parent candidates, as many as young animals can be first selected based on genetic markers, and then selected groups of parents progeny-tested; 3) decrease the generation interval by elimination of the progeny test. Moreover, MAS will be particularly useful for the improvement of traits which are sex limited such as milk production by analyzing the DNA information from males, traits which are expensive to measure such as feed intake, or traits which can only be measured after animals are slaughtered such as body composition (Buchanan et al., 1993).

CHAPTER III

PLASMA CHOLECYSTOKININ-8 DURING FEEDING IN PIGS WITH DIVERGENT GENETIC POTENTIAL FOR FEED INTAKE AND GROWTH

Abstract

Plasma concentrations of cholecystokinin-8 (CCK-8) in response to feeding were studied in lines of pigs with divergent genetic potential for feed intake and growth. Differences in feed intake between the lines resulted from ten generations of divergent selection for either fast (line F) or slow (line S) growth when both lines were fed ad libitum. Two experiments were conducted to test the hypothesis that the lower feed intake in S than in F may be due in part to greater plasma concentrations of the satiety hormone CCK-8 in S pigs both in the fasted and fed state. In experiment 1, F (n=23) and S (n=19) barrows were fed maintenance to attain zero growth at 90 kg body weight and were used to determine plasma CCK-8 concentrations in response to ad libitum feed consumption independent of differences in growth rate. Blood samples were collected via jugular catheter in overnight fasted pigs at 30, 15, and 1 minute before feeding (0800 hr) and at 10, 20, 30, 60, 90 and 120 minutes after feedings. As expected, barrows from F ate more than those from S when allowed ad libitum access to feed ($P<0.05$). Averaged across sampling times, mean concentrations of CCK-8 tended to be greater ($P = 0.07$) in S (7.63 pg/ml) than in F (5.76 pg/ml). In experiment 2, plasma CCK-8 concentrations were

measured for pairs (F, S; n = 9) of the same barrows in experiment 1 when the intake of each pair was standardized to that of the S pig during experiment 1. Blood samples were collected as in experiment 1. Averaged across times, the difference between CCK-8 concentrations of S (13.28 pg/ml) and F (9.05 pg/ml) barrows was not significant (P = 0.18). A line x time interaction was not detected in either experiment. Overall, these results indicate a tendency for greater concentration of CCK-8 in S than in F barrows and suggest that CCK-8 may play a role in genetic differences between the lines for feed intake.

Introduction

Feed intake is one of the major factors that determine profitability in the swine industry. A better understanding of the genetic basis of appetite may lead to the development of more effective selection methods for feed efficiency. Although the general physiological processes that result in hunger and satiety have been the focus of extensive research (Morley, 1995; Kaiyala et al., 1995; Figlewicz et al., 1996), the molecular nature that contributes to genetic variation in appetite remains largely unknown. Genetic variation in appetite originates from differences in genes that encode the peptides/proteins involved in the feeding control pathways. Investigation of populations that differ only in their genetic propensity to eat may lead to identification of the relevant physiological pathways that control appetite, and aid in the search for the specific genes involved.

Divergently selected lines of pigs have been studied to understand genetic variation in obesity (Wangsness et al., 1981). However, this approach has not been used to identify a relationship between genetic variation and mechanisms of appetite regulation. Our laboratory has two lines of pigs that have been undergone ten generations of divergent selection for either fast or slow postweaning average daily gain (ADG). Woltmann et al. (1992) reported that after four generations, barrows from the lines differed in gain (0.16 kg/d difference) and feed intake (0.41 kg/d difference) when allowed ad libitum access to feed, but had similar gains when feed intake was standardized. They concluded that most of the difference in gain was expressed through a correlated genetic response in feed intake. Subsequent evaluations of the lines through seven generations of selection revealed that gain and feed intake have continued to diverge between the lines (Woltmann et al., 1995; Clutter et al., 1995).

Consequently, a study was conducted to determine the physiological basis for the observed genetic differences in feed intake between the lines. The peptide hormone cholecystokinin (CCK) has been reported act as a satiety signal in several species including pigs (Anika , 1981; Della-Fera et al., 1979; Micelli et al., 1983; McLaughlin et al., 1985; Denbow et al., 1982; Morley, 1985, 1995; Silver et al., 1990; Reidelberger, 1994). The objective of the present investigation was to determine if plasma concentrations of an eight amino-acid molecular form of the putative hormone CCK (CCK-8) differed between the lines during feeding. The hypothesis was that the S line pigs have less appetite and relatively greater circulating concentrations of CCK-8.

Materials and Methods

Selection Lines and Sampling of Lines

A study was started in 1979 to investigate direct and correlated response to divergent selection for ADG. Detailed descriptions of the base population, animal care and selection procedures have been reported previously (Woltmann et al., 1992, 1995; Clutter et al., 1995). In the first year, Hampshire boars were purchased in pairs from central test stations in Iowa, Missouri, Nebraska and Oklahoma. Boars were evaluated on the index recommended by the National Swine Improvement Federation that emphasized increased ADG, decreased BF, and improved feed efficiency as described by Woltmann et al. (1992). In each pair, one boar had a high index value (index value $\geq$ 118) and the other a low index value (index value $<$ 90). These boars were mated to a population of Duroc $\times$ Yorkshire $\times$ Landrace $\times$ Spotted crossbred gilts from a previous study (Woltmann et al., 1992). In the second year, pairs of Duroc boars were purchased by the same criteria. Duroc boars were mated with females produced in the first year. Line F was generated from pigs sired by high-indexing Duroc boars and out of gilts sired by high-indexing Hampshire boars. Line S was derived from pigs sired by low-indexing Duroc boars and out of gilts sired by low-indexing Hampshire boars. The lines were then closed and have undergone ten generations of selection for either fast (F) or slow (S) ADG from 9 wk of age to 100 kg. The selection lines were replicated in spring- and fall-farrowing groups. The spring replicate farrowed during mid-March through April, and the fall replicate farrowed during mid-September through October. Complete replacement of boars and gilts resulted in a generation interval of 1 year. Selection was relaxed after 10

generations. Animals for the present experiments were sampled from F and S litters in the final set of progeny from selected parents (spring 1993), and the first two consecutive sets under relaxed selection (fall 1993 and spring 1994).

Barrows were chosen for the present study so that potential variation due to sexual activity would be avoided and because the most extensive data previously collected in the lines for feed intake were from barrows (Woltmann et al., 1992). Each barrow was sampled from a different litter. The litter began postweaning test at 8 weeks of age according to the routine procedures implemented in the reference population. When a litter reached the approximate average weight of 75 kg, the barrow closest to the average weight was chosen so that the candidate barrow from each litter weighed approximately 90 kg at data collection. The candidate barrows were penned in groups of 4 to 6 in a room before they were brought into the experimental rooms.

Experimental Procedures

Barrows chosen for the experiments were housed individually in pens (1.8 m^2) in rooms with an average temperature of 23 ± 3 ^{0}C and subject to a 12 hr light : 12 hr dark cycle. During an initial acclimation period, barrows were allowed ad libitum access to feed (Table 1) except for removal of feed and feed troughs between 2000 hr and 0800 hr each day. After this period of acclimation, barrows were fed amounts intended to be sufficient only for maintenance of body weight (zero growth) for a minimum of 14 d before the start of experiment 1. This was done so that plasma CCK-8 in response to feed intake could be compared in F and S barrows without confounding due to differences in growth rate. During feeding for zero growth, barrows received approximately 8.5 g feed/kg body weight at 0800 hr and 1800 hr daily. The average growth rate of barrows

Table 1. COMPOSITION OF DIET USED IN THE STUDY

Ingredient	Percentage [a, b]
Corn	82.40
Soybean meal (445 CP)	14.63
Dicalcium phosphate	1.50
Calcium carbonate	0.82
Salt	0.40
Vitamin-trace mineral	0.25

[a] As-fed basis
[b] Balanced to 0.75% lysine.

during the week before experiment 1 was 0.09 kg/d, and did not differ between the F and S groups. Thus, the difference in growth rate between barrows from F and S was successfully removed for the present experiments. Polyvinyl catheters were inserted into the jugular vein of anesthetized barrows 48 hr before experiment 1 began. Catheters were tunneled subcutaneously and exteriorized on the dorsal midpoint of the neck. The distal end of the exteriorized catheter was fitted with a luer stub adapter, capped and placed in a protective packet that was glued to the skin. Catheter length allowed blood sampling from outside the pen and thus prevented interference in normal feeding behavior during the experiments. Feeding for zero growth was continued during the 48 hr before experiment 1. Blood collected during feeding was discarded, in order to acclimate barrows to the experimental conditions.

In experiment 1, barrows sampled from F (n=23) and S (n=19) were used to determine the plasma CCK-8 in response to unrestricted feed intake. On the morning of the experiment, blood samples (10 ml) were collected into heparinized syringes at 30, 15, and 1 min before and 10, 20, 30, 60, 90 and 120 min after introduction of feed and the beginning of feeding at 0800 hr. Blood was transferred immediately into tubes held in an ice-water bath.

To measure individual feed intakes, feed (2.0 kg) was weighed to the nearest 1.0 g and placed in each of six feed troughs 1 hr before the beginning of the experiment. The first trough containing feed was placed in the pen at time 0 and troughs were removed and replaced with a fresh trough of feed at 10, 20, 30, 60, and 90 min. Feed intakes were determined as the difference in weight of feed in each trough before and after its placement in the pen. Feed intakes were measured within 1 min of blood sample collections.

Experiment 2 was conducted 48 hr after experiment 1. Feeding for zero growth was maintained during the 48 hr between experiments 1 and 2. Experiment 2 determined the plasma CCK-8 response to feed intake when intakes were equally matched in pair-fed F and S barrows. Barrows from F and S were paired randomly and each barrow within a pair was allocated an amount of feed equal to the ad libitum intake of the S barrow in that pair as measured in experiment 1. Blood samples were collected and feed intakes determined as described for experiment 1. A total of 9 pairs of barrows were evaluated in experiment 2.

Radioimmunoassay Protocol and Validation

Plasma Extraction: For each sample, 0.7 ml of plasma was extracted with 1.4 ml of 100% ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY). Samples were placed at room temperature for 30 min after vigorous vortexing for 10 sec. The mixture was centrifuged for 30 min (1,500 × g) at 4 °C. The supernatant was transferred into a clean 12 × 75 mm polypropylene tube and then evaporated in Speed Vac Concentrator (SVC-100H, Savant Instruments Inc., Farmingdale, NY) at room temperature. The dried extracts (unreconstituted) were stored at –20 °C until assayed.

Radioimmunoassay Procedure: Dried samples were reconstituted to original volume with assay buffer (pH 8.5) containing 20 mmol/l Barbital, 0.05% sodium azide, 0.11% gelatin (all reagents from Sigma Chemical, St. Louis, MO) at least one hour before use. Cholecystokinin octapeptide (CCK-8; [Tyr(SO_3H)^{27}]-Cholecystokinin Fragment 26-33 Amide, Sigma Chemical, St. Louis, MO) was dissolved in distilled water and diluted with assay buffer to produce CCK-8 standards. Reconstituted plasma extract (200 µl) or CCK-8 standards (0, 1, 5, 10, 20, 40, 80, and 120 pg/ml) were added to 12 × 75 mm polypropylene assay tubes. Rabbit antiserum (NO. 92128; kindly provided by Dr. J.F. Rehfeld, University Hospital, Copenhagen, Denmark) specific for tyrosine O-sulfated and phenylalanine α-amidated CCK-peptides was added (200 µl) to all tubes except Total Counts and NSB in an initial dilution 1 : 12,500 (a final solution of 1: 50,000), resulting in an initial binding of labeled hormone of approximately 34%. Assay buffer (200 µl) was added to NSB tubes, and then 300 µl of assay buffer to all tubes except Total Counts. Tubes were incubated 24 to 36 hours at 4 °C. One-hundred µl of [^{125}I]-CCK-8 (Amersham Corporation, Arlington Hts, IL) containing 3000-5000 cpm in assay buffer was added to all tubes. Tubes were incubated overnight at 4 °C. One milliliter of cold

charcoal/dextran (0.75% dextran, 3.00% charcoal, Sigma Chemical, St. Louis, MO) was added to all tubes except Total Counts to separate antibody-bound and free [^{125}I]-CCK-8. Supernatant was aspirated after centrifuging at 1,500 × g for 10 min at 4 °C. Charcoal-precipitate was counted in an automatic gamma-counter (Micromedic systems, Inc., Horsham, PA) and concentrations of CCK-8 in standards and unknown samples were calculated by an iterative least-squares regression on percentage binding.

Validation of CCK-8 Radioimmunoassay: The sensitivity of the assay, defined as the smallest amount of CCK-8 which could be differentiated from zero hormone concentration with 95% confidence, was 1.4 pg/ml. Accuracy, defined as the extent to which measurements of CCK-8 agreed with the exact amount present in a sample, was evaluated by adding known amounts of purified synthetic CCK-8 to plasma. When known amounts of synthetic CCK-8 were added to pig plasma samples (n=6) before extraction, a correlation coefficient (r) of 0.99 was associated with the recovery curve (Figure 1). Essentially 100% of the added CCK-8 was recovered by the assay after subtraction of the endogenous hormone. Intra-assay precision was determined by calculating the coefficient of variation (CV) of 3 pools of pig plasma containing low (L.P.), medium (M.P), and high (H.P.) concentration of CCK-8 when each was run seven times in one assay (Table 2). Inter-assay precision was determined by calculating the CV of 3 pools of pig plasma (L.P., M.P., and H.P.) estimated in 10 separate assays (Table 2). The inhibition curves produced by the standard solution of CCK-8 and serial dilutions of pig plasma pools are shown in Figure 2. Parallel inhibition curves demonstrated an unique combination of antibody and antigen and indicated that the same substance in standard solutions and plasma was being measured.

Statistical Analyses:

Methods of least-squares analyses used for data from both experiments 1 and 2 were as follows. Plasma concentrations of CCK-8 at each of the times relative to introduction of feed (–30, –15, –1, 10, 20, 30, 60, 90 and 120 min), as well as cumulative feed intake at 10, 20, 30, 60, 90 and 120 min after introduction of feed, were analyzed with a model that included the effects of farrowing group (spring 1993, fall 1993, or spring 1994), line, the farrowing group × line interaction, animal within farrowing group × line, time and all interactions of time with line and farrowing group. Effects of farrowing group, line and the farrowing group × line interaction were tested using animal

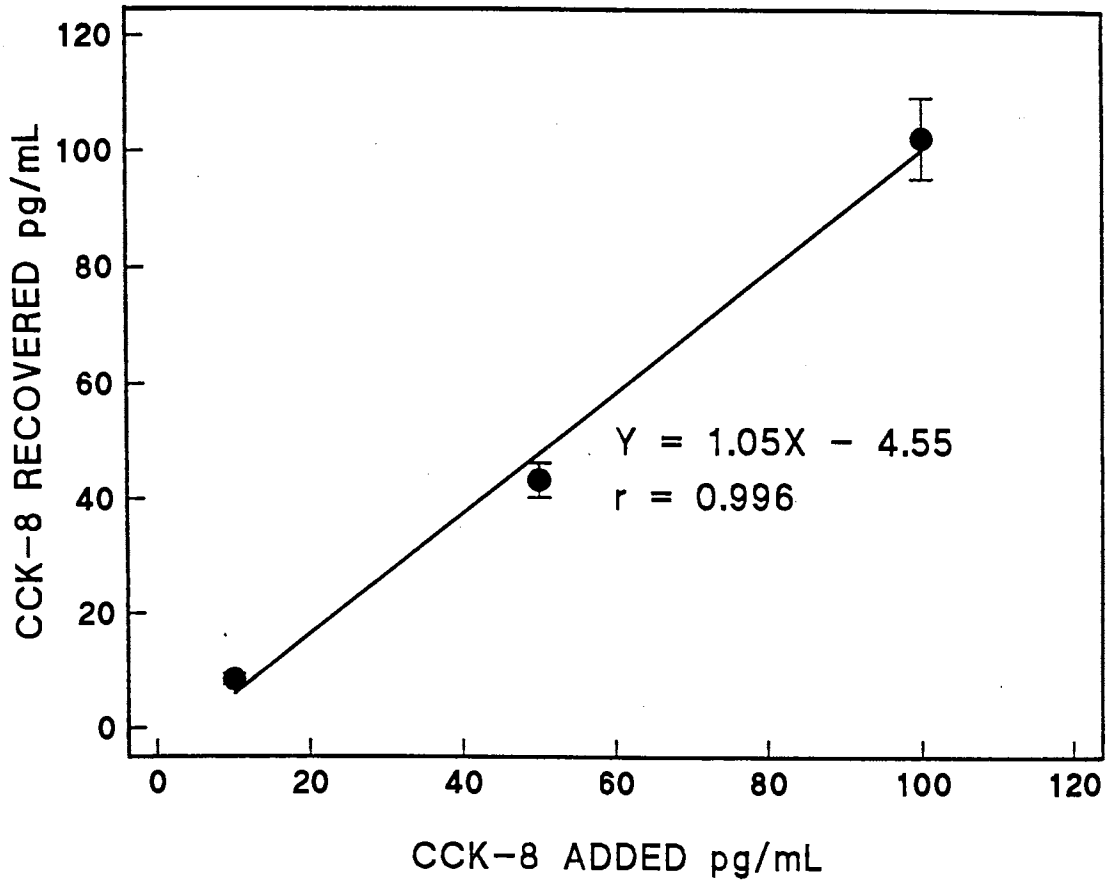

Figure 1. Recovery of CCK-8 when known amounts of synthetic CCK-8 were added to pig plasma samples before samples were extracted (see methods) (n=6).

Table 2. PRECISION IN THE CCK-8 RADIOIMMUNOASSAY

Precision	Pool	N	Concentration (pg/ml)	CV (%)
Intra-assay	L.P.	7	6.81 ± 0.46	10.9
	M.P.	7	21.15 ± 1.52	19.0
	H.P.	7	60.40 ± 5.04	18.6
Inter-assay	L.P.	10	7.68 ± 0.54	22.0
	M.P.	10	19.83 ± 0.88	14.0
	H.P.	10	42.55 ± 1.70	12.6

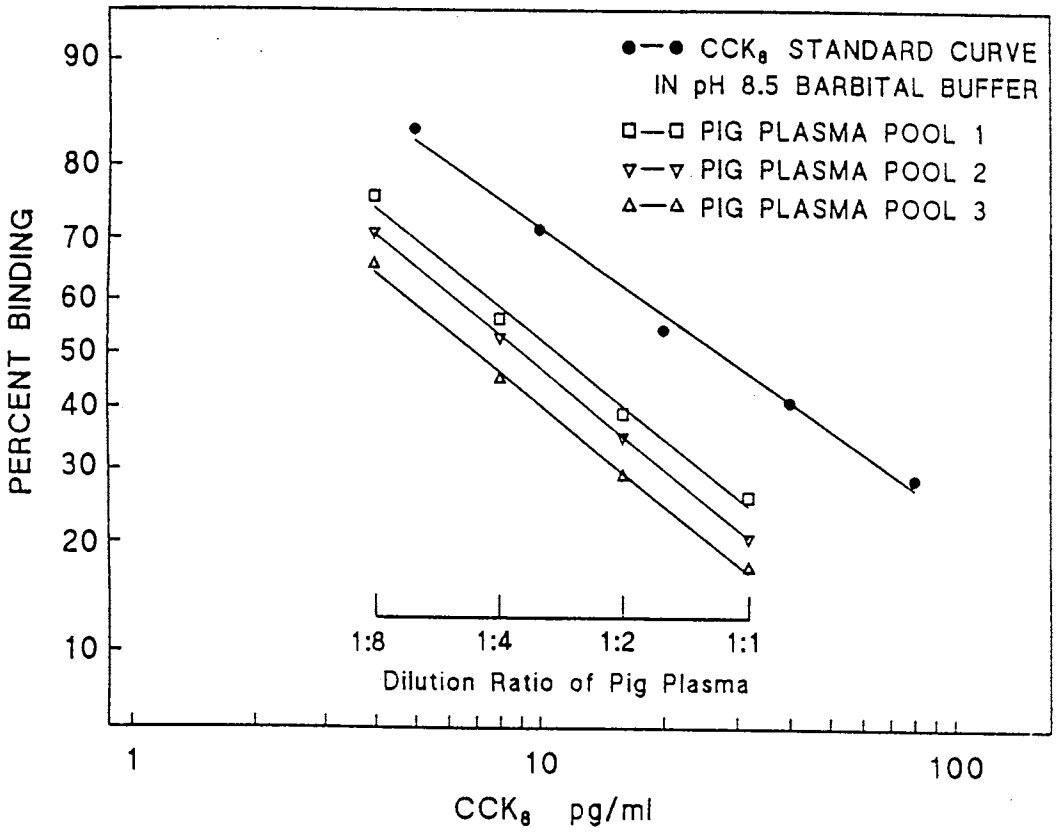

Figure 2. Parallelism in the CCK-8 radioimmunoassay.

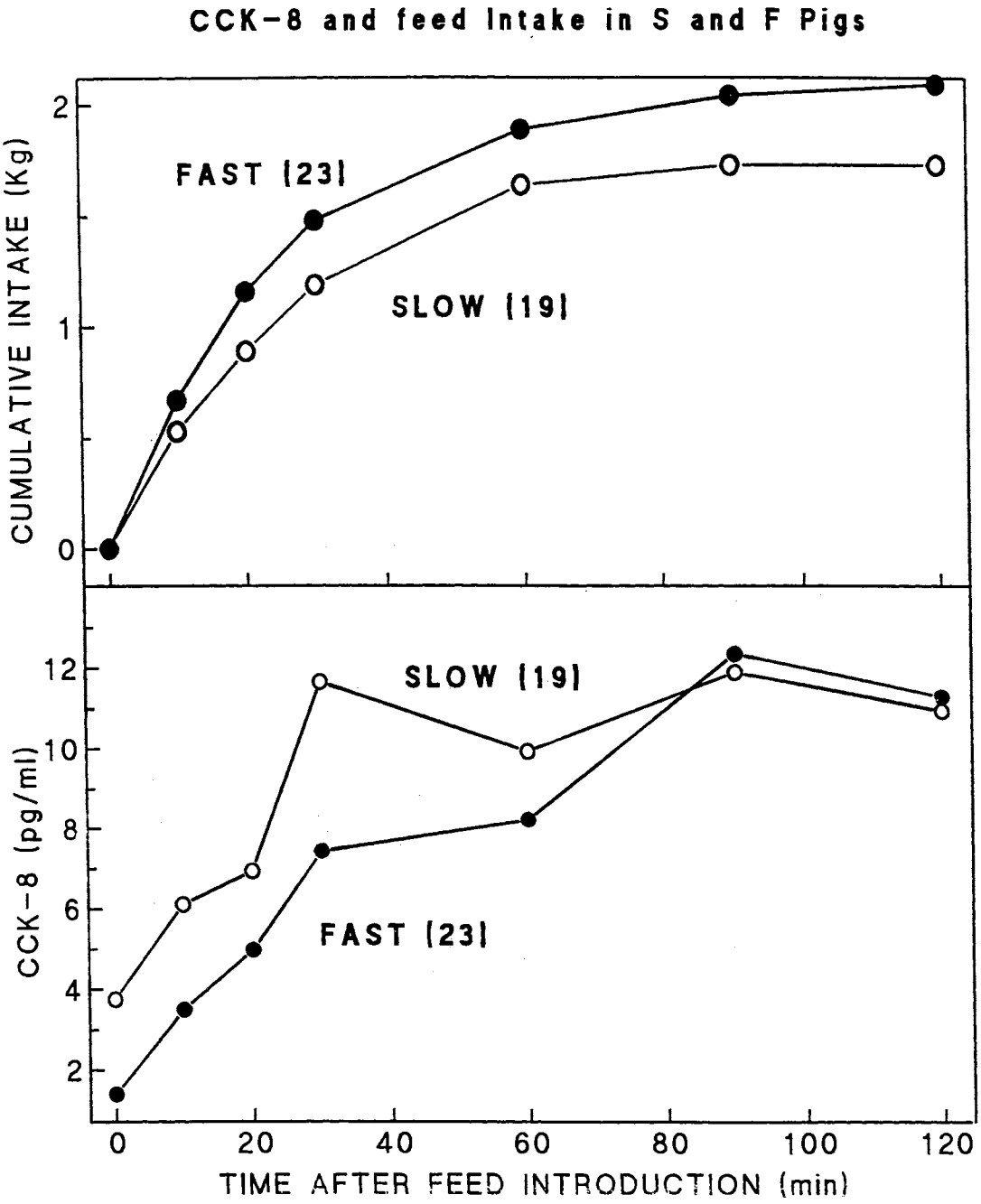

Figure 3. Cumulative feed intake of barrows and plasma concentrations of CCK-8 in barrows from the F and S lines during experiment 1. Barrows were at zero growth, fasted overnight and were allowed ad libitum access to feed for 2 hours. Concentrations of CCK-8 at time 0 were calculated using the average of 3 observations in each pig.

times, concentrations of CCK-8 tended to be greater (P = 0.07) in S barrows (7.63 pg/ml) than in F barrows (5.76 pg/ml). A time × line interaction was not detected.

Results of the analyses of basal CCK-8, response in CCK-8 and total CCK-8 are presented in Table 3. Basal CCK-8 tended (P = 0.09) to be greater in S than in F. However, neither response in CCK-8 nor total CCK-8 differed between the lines.

Experiment 2. Mean values for cumulative feed intake in experiment 2 are plotted in Figure 4. Cumulative feed intakes did not differ significantly between F and S barrows at any of the measurement times. Thus, the objective to constrain F and S barrows to an equally matched feed intake was achieved.

Table 3. LEAST-SQUARES MEANS FOR BASAL CCK-8, RESPONSE IN CCK-8 AND TOTAL CCK-8 CALCULATED FROM THE AREA UNDER EACH CURVE[a].

	CCK-8 (pg/ml · min)		
	Basal	Response	Total
Experiment 1[b]			
F	189.13 ± 35.87[d]	894.93 ± 126.07	1084.11 ± 149.50
S	514.29 ± 181.16	796.92 ± 207.34	1311.22 ± 195.87
Experiment 2[c]			
F	551.47 ± 158.07	877.34 ± 173.14	1428.81 ± 255.29
S	726.93 ± 102.23	1361.39 ± 371.7	2088.32 ± 414.23

[a] Trapezoid method.
[b] Barrows had ad libitum access to feed after overnight feed deprivation.
[c] Barrows pair-fed (F, S) amounts equal to the ad libitum intake in experiment 1 of the S barrow in the pair.
[d] Least-square mean ± SE.

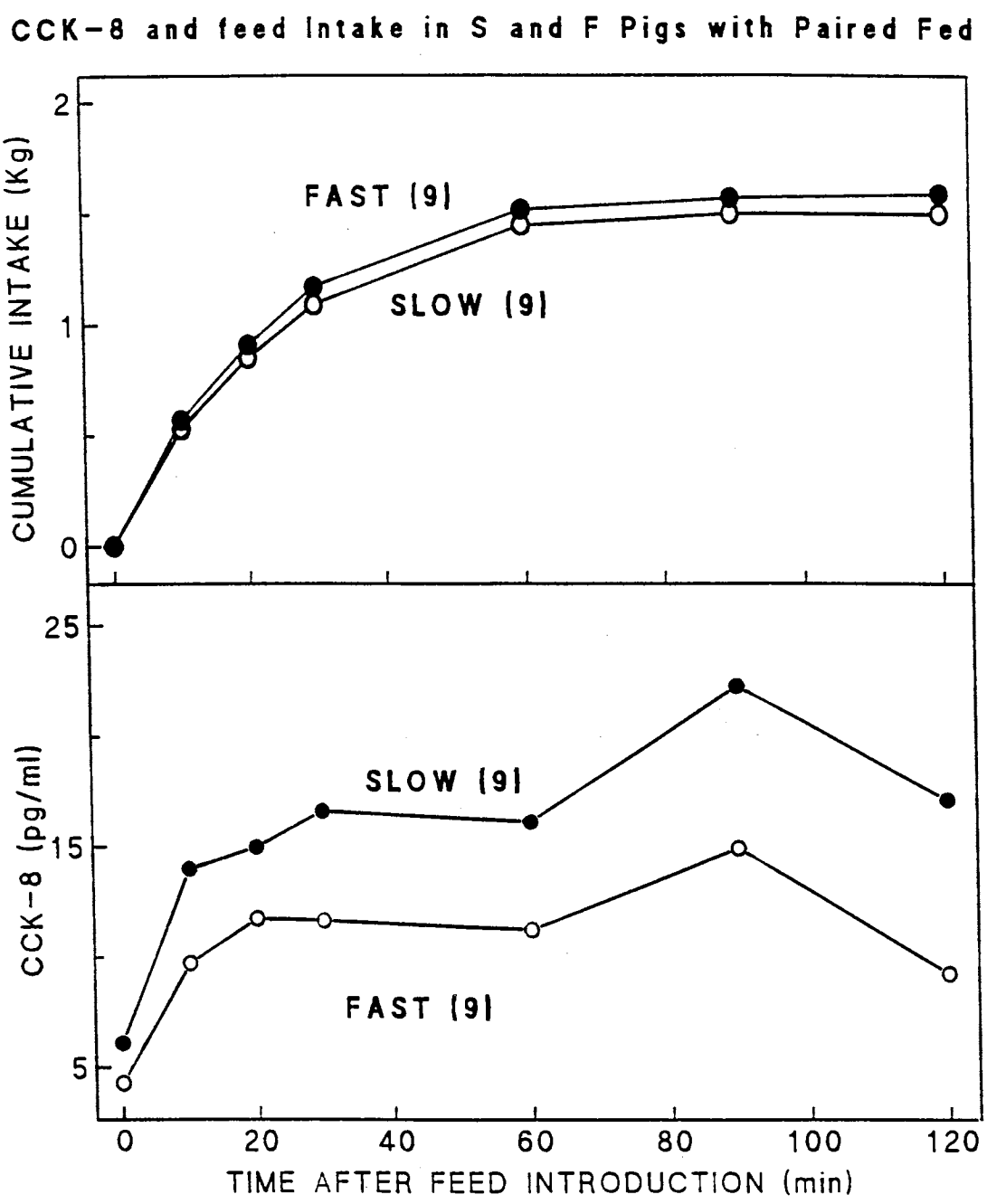

Figure 4. Cumulative feed intake of barrows and plasma concentrations of CCK-8 in barrows from the F and S lines during experiment 2. Barrows were at zero growth, fasted overnight and F pigs were pair-fed amounts equal to the previous ad libitum intake of the S barrow in each pair. Concentrations of CCK-8 at time 0 were calculated using the average of 3 observations in each pig.

Least-squares means from the analysis of individual CCK-8 concentrations are also plotted in Figure 4. As in experiment 1, concentrations of CCK-8 in both lines increased ($P < 0.01$) after feeding began. Averaged across time, the difference between CCK-8 concentrations of S (13.28 pg/ml) and F (9.05 pg/ml) barrows was not significant ($P = 0.18$). A time × line interaction was not observed.

Each of the mean values for area under the CCK-8 response curve was numerically greater for S than for F barrows (Table 3), but no significant difference was detected.

Discussion

The reports of the selection lines from different generations revealed a continued divergence in daily feed intake and ADG between F and S lines (Woltmann et al., 1992, 1995; Clutter et al., 1995). Barrows from F and S had similar ADG when feed intake was standardized , leading to the conclusion that most of the difference in ADG was exhibited through a correlated genetic response in feed intake (Woltmann et al., 1992). Those results from the selection lines were in agreement with reports of direct and correlated responses to selection for growth in other populations of pigs (Baird et al., 1952; Fine et al., 1953; Kuhlers et al., 1992). In other words, the selection for ADG has targeted DNA sequences which encode proteins involving the feeding control pathways.

The regulation of feed intake involves many physiological factors (Morley, 1995; Kaiyala et al., 1995; Figlewicz et al., 1996) which, therefore, are candidate sources of genetic variation in feed intake. Many peptide hormones have been shown to have effect

on feeding (for example. CCK, glucagon, bombesin, leptin, insulin, and NPY). Among these peptides, however, one of the best-characterized satiety peptides is CCK (Morley, 1995). The hypothesis that CCK functions as a satiety signal was initially based on the finding that systemic injection of CCK reduced food intake in rats (Gibbs et al., 1973). Since then, evidence from studies of both agonists and antagonists has supported this hypothesis in many species including pigs (Morley, 1987, 1995; Crawley et al., 1994; Reidelberger, 1994).

CCK is encoded by a single gene (Deschenes et al., 1985) and synthesized via the preprohormone pathway. PreproCCK consists of 114 amino acids in pigs (Gubler et al., 1984) and 115 amino acids in man (Takahishi et al., 1985) and rats (Deschenes et al., 1984). Different molecular forms of CCK containing 58, 39, 33, 22, 12, 8, or 4 amino acids have been identified in tissues and blood (Cantor,1989; Crawley et al., 1994). In pigs, Cantor and Rehfeld (1989) observed that CCK-22-like immunoreactivity accounted for 42% of all CCK immunoreactivity in peripheral plasma after intraduodenal infusion of HCI, but also reported lesser and equal amounts (24%, respectively) of CCK-33- and CCK-8-like forms. Exogenous CCK-8 inhibits feed intake in a variety of species (Della-Fera et al., 1979; Joyner et al., 1993; Covasa et al., 1994) including pigs (Anika et al., 1981; Baldwin et al., 1982). Agonists for CCK-A receptors decreased feed intake in pigs (Parrott, 1992). Specific antagonists for CCK-A receptors (Ebenezer et al., 1990; Baldwin et al., 1992, 1994), and specific immunoneutralization of circulating CCK-8 (Pekas et al., 1993), have been shown to increase feed intake in pigs. Thus, CCK has been established as a satiety hormone in the pig and CCK-8 was chosen as the focus of the present experiments.

In both experiments 1 and 2, the general patterns of CCK-8 concentrations in response to feeding were similar to those reported for humans (Cantor, 1989) and pigs (Cantor et al., 1989) in which the assays detecting bioactive forms of CCK were used. Cantor (1989), in summarizing reports of CCK in human plasma, concluded that basal concentrations of CCK-8 averaged 1 to 2 pM/L (or 1.1 pg/ml to 2.3 pg/ml) and increased from 6 to 8 pM/L (or 6.8 pg/ml to 9.1 pg/ml) after a meal. Cantor and Rehfeld (1989) reported basal concentrations of CCK-8 approximately 1 pM/L (or 1.1 pg/ml) in the pig that increased to 12.3 pM/L (or 14.1 pg/ml) by 10 min after intraduodenal infusion of HCI.

In experiment 1, CCK-8 concentrations increased following the introduction of feed, and seemed to reach a plateau after 30 min in S and after 90 min in F (Figure 3). Thus, the tendency for a greater concentration of CCK-8 in S than in F prior to feeding was maintained through the first hour after the introduction of feed. The slightly contrasting CCK-8 patterns in S and F at the end of the experimental period are consistent with the continued feed consumption of F barrows from 60 to 90 min after the introduction of feed.

In experiment 2, numeric differences between CCK-8 concentrations of S and F barrows were greater than in experiment 1, but can only be considered significant with 15 to 20% probability of a Type I error. Comparison of the CCK-8 patterns (Figure 4) suggests that following the introduction of feed, concentration of CCK-8 tended to increase at a greater rate during the first 10 min of feeding in S than in F, after which the patterns were very similar for the two lines. The similarity of CCK-8 patterns between S and F during the latter part of the experimental period is consistent with the pair-feeding

design of experiment 2 and, on the average, the limitation of feed intake by F barrows relative to experiment 1.

Taken together, these results indicate a tendency for greater plasma concentrations of CCK-8 in S than in F barrows and support the hypothesis that selection has changed feed intake, in part, by altering circulating CCK concentrations. Although the assay accurately measured concentrations of CCK-8, adequate statistical power for comparisons of this type is generally difficult to achieve due to inherent genetic variation among individuals within a selection line. Thus, detection of differences between the lines when allowed a standard feed intake (experiment 2) was probably inhibited by the relative low power associated with observations on only 18 total pigs.

The assay procedures implemented here were specific for CCK-8. Although the importance of CCK-8 as a satiety signal in the pig has been implied through infusion studies (Anika et al., 1981; Baldwin et al., 1982) and the specific immunoneutralization of CCK-8 (Pekas et al., 1993), the relative effects of bioactive forms of CCK on feeding behavior remains to be determined. The effect of selection on relative changes of molecular forms of CCK in the present experiments can only be determined by additional work to separate peptide fractions (HPLC/RIA). In addition, experiments to determine the effects of CCK infusion, CCK receptor antagonists and CCK immunoneutralization on feed intake in F and S may provide a more complete picture of the role of CCK action in genetic differences between the lines.

Reports of physiological differences between selection lines are relative rare (Goddard et al., 1988; McKnight et al., 1989; Arbona et al., 1992; Hastings et al., 1993), especially in large-animal species (Arbona et al., 1988; Norton et al., 1989). To our

knowledge, studies of CCK in different genetic strains have been limited to *ob/ob* mice (Straus et al., 1979) and Zucker obese rats (McLaughlin et al., 1985; Moos et al., 1982). In each case the inheritance of obesity is due to a single gene mutation. Straus and Yalow (1979) reported that the CCK content in brains of *ob/ob* mice was lower than in the brains of their *OB/-* (lean) littermates. Zucker obese rats expressed greater hypothalamic concentrations of CCK during the feeding period, but also consumed more feed than controls (McLaughlin et al, 1985). Moos et al. (1982) reported that Zucker obese rats expressed less sensitivity to satiety effects of CCK than their control littermates. Characteristics of CCK action associated with quantitative genetic differences between selected lines of animals have not been reported.

Direct and correlated responses to selection are due to changes in the frequencies of gene forms (alleles) affecting each of the measured traits (Falconer, 1989). Although differences between divergent lines may be due in part to founder effects in the establishment of the lines or random changes in gene frequencies due to finite population size (Falconer, 1989), the primary factor affecting gene frequencies in F and S was selected for ADG. Therefore, phenotypic and physiological differences between the lines are likely due to differences in the frequencies of alleles targeted by selection. Because of the complex and quantitative nature of these traits, selection for ADG, and indirectly for feed intake, has no doubt targeted the alleles of many genes.

A previous study of F and S (Clutter et al., 1995) revealed greater plasma IGF-I and lesser IGF binding proteins in F than in S, a relationship consistent with the relative growth rates of the lines. As those authors discussed, the results indicated the IGF pathway was an important mechanism of response to selection, but the degree to which

selection targeted the IGF and IGF binding protein genes versus other genes that affect growth through IGF was not discernible. In addition, because S and F pigs were measured during full expression of their growth potential, the physiological differences observed were confounded by the differences in growth rate.

The design of experiment 1 and 2 of the present study included the condition of zero growth to avoid the confounding influence of different growth rates between the lines, and experiment 2 was designed to remove confounding due to differences in feed intake. However, as discussed by Clutter et al. (1995), these physiological evaluations of selection lines only provide a picture of the genetic differences at the protein level. If, in fact, concentrations of CCK-8 are different in F and S, it is impossible to determine from these comparisons between the lines if selection has acted on the CCK gene directly, on genes that code for enzymes involved in the posttranslational modifications of the preproCCK, or other genes that result in altered CCK concentrations.

The availability of markers in anonymous DNA and functional genes from throughout the porcine genome (Archibald et al., 1995; Rohrer et al., 1995), including a marker for the CCK gene (Clutter et al., 1996), provides the opportunity to identify chromosomal regions and eventually the specific genes that contribute to genetic variation in growth and its components feed intake and efficiency. The successful application of these markers to detect important loci affecting traits of interest requires a population in which the relevant alleles are segregating at those loci (Lander et al., 1989). An example of such a resource population is an F2 cross originating from grandparent stock that is phenotypically divergent for the traits of interest. The phenotypic and physiological divergence detected between the F and S lines in the present and previous

studies (Woltmann et al., 1992, 1995; Clutter et al., 1995) suggests the lines as candidates to produce a resource family in which loci contributing to variation in feed intake and growth can be identified.

In summary, results from the present experiments revealed a tendency for greater plasma concentrations of CCK-8 in S than in F, and support the hypothesis that the line has been selected for slow growth, and that exhibits less appetite than the line selected for fast growth, has relatively greater circulating concentrations of CCK-8. Studies of the effects of infusion of exogenous CCK, CCK receptor antagonists, or CCK immunonuetralization on feed intake may provide a more complete picture of differences in CCK action between these selected lines.

Although the results suggest that genetic differences in appetite between the lines are reflected in part by differences in CCK action, the relative contributions of individual genes or chromosomal regions to variation in appetite can only be determined in families in which the important gene forms are segregating. The F and S lines may be useful as parent or grandparent stock in the production of such a family.

CHAPTER IV

FEEDING RESPONSE TO EXOGENOUS CHOLECYSTOKININ-8 IN PIGS WITH DIVERGENT GENETIC POTENTIAL FOR FEED INTAKE AND GROWTH

Abstract

The objective was to determine the effects of an eight amino acid molecular form of the satiety hormone cholecystokinin (CCK-8) on feed intake in lines of pigs with divergent genetic potential for feed intake and growth. After ten generations of selection for either fast (F) or slow (S) postweaning gain (ADG), overall daily feed intake and ADG were 36% and 47% greater in F than in S, respectively. It was hypothesized that the lower feed intake in S relative to F may be partly due to a greater sensitivity to feeding-induced increases in CCK-8 in S than F pigs. Barrows from F and S restricted to zero growth during the experimental period were infused iv with 0 (saline), 60, 100, or 300 ng/kg sulfated CCK-8 immediately before they were allowed ad libitum access to feed for 2 hr. Individual feed intakes were determined at 10, 20, 30, 60, 90 and 120 min after introduction of feed. Blood samples were collected via jugular catheter at 30, 15, and 2 min before and 10, 20, 30, 60, 90 and 120 min after introduction of feed. Plasma concentrations of CCK-8 were similar ($P > 0.30$) in F and S at each dosage of CCK-8. Feed consumption was reduced in both lines as dosage of CCK-8 increased, but the reduction at each dose was greater in S than F ($P < 0.01$). The dose of CCK-8 needed to

inhibit intake by 25% or 50% was less ($P < 0.05$) in S (D_{25} = 56.6 ng/kg; D_{50} = 146.47 ng/kg) than in F (D_{25} = 94.9 ng/kg; D_{50} = 745.58 ng/kg) pigs. These results indicate that the genetic difference between these lines for appetite may be due in part to a difference in sensitivity to the CCK-8 satiety signal.

Introduction

The peptide hormone CCK is synthesized via the preprohormone pathway and exists in different molecular forms. To date, 58, 39, 33, 22, or 8 amino acid forms have been identified in tissues and blood of pigs (Crawley et al., 1994). The C-terminal sulfated octapeptide sequence, Aps-Tyr(SO_3H)-Met-Gly-Try-Met-Asp-Phe-NH_2 (CCK-8), is relatively conserved across species. CCK-8 appears to be the minimum sequence necessary for the biological activity (Crawley et al.,1994) and the most biologically potent form of CCK for the satiety effects (Reidelberger, 1994). The hypothesis that CCK functions as a satiety signal was first based on the reduction in feed intake of rats that received systemic injections of CCK (Gibbs et al., 1973). Since then, physiological evidence from studies of both agonists and antagonists have supported this hypothesis in many species (Morley, 1985, 1995; Silver et al., 1990; Baldwin et al., 1992; Parrott, 1993). Agonists for CCK-8 or CCK-33 decreased feed intake (Baile et al., 1981; Morley, 1985, 1995) and antagonists for CCK-8 or CCK-33 increased feed intake (Baile et al., 1981; Baldwin et al., 1992; Ebenezer et al., 1990; Silver et al., 1990). Exogenous CCK-8 inhibits feed intake in a variety of species including sheep (Della-Fera et al., 1979), rats (Joyner et al., 1993), mice (Silver et all., 1989), goldfish (Himick et al., 1994), baboons (Figlewics et al., 1992), chickens (Covasa et al., 1994), humans (Kissileff et al., 1981)

and pigs (Anika et al., 1981). Intravenous injection of CCK-8 (Anika et al., 1981; Baldwin et al., 1982) and CCK-A agonists (Parrott, 1993) decreased feed intake in pigs. Specific antagonists for CCK-A receptors (Ebenezer et al., 1990; Baldwin et al., 1992, 1994) and immunoneutralization of circulating CCK-8 (Pekas et al., 1993) also have been shown to increase feed intake in pigs.

Studies of the genetic basis for the relationship between CCK and appetite regulation have been limited to obese rodent strains. The total CCK content of brains of *ob/ob* mice was lower than that of their *OB/-* contemporaries (Straus et al., 1979). Zucker obese (*fa/fa*) rats expressed greater total CCK concentrations in hypothalamus during feeding, but lesser sensitivity to satiety effects of CCK (Moos et al., 1982). OLEFT rats increased CCK-8 concentrations in plasma during feeding, but failed to response to satiety effects of exogenous CCK-8 (Miyasaka et al., 1994). In each case the inheritance of obesity is due to a single gene effect. However, the relationship between normal genetic variation in feed intake and CCK has not been reported.

Our laboratory has lines of pigs that have been undergone ten generations of divergent selection for either fast (F) or slow (S) postweaning average daily gain (ADG). Evaluation of results from the generation ten revealed that F pigs ate more (36% per day) and grew faster (47% per day) than S pigs (Clutter et al., 1997). Less appetite in S than in F may be in part due to more satiety effects of CCK-8 in S. This may be caused either by greater plasma CCK-8 in S than F or by changed/unchanged plasma concentrations of CCK-8 together with greater cellular response (i.e., satiety) to CCK-8 activation of CCK receptors involved with regulation of appetite in S than in F. Results reported in Chapter III indicated greater plasma concentrations of CCK-8 in S than in F barrows at the α level

of 0.07 and suggested that CCK satiety action may play a role in genetic differences between the lines for feed intake. The objective of present investigation was to determine if pigs with divergent genetic potential for feed intake differ in feed consumption in response to infusion with exogenous CCK-8. The hypothesis was that the genetic differences for appetite between the lines is due in part to differences in sensitivity to the CCK-8 satiety signal.

Materials and Methods

Selection Lines and Sampling of Lines

A study was initiated in 1979 to investigate direct and correlated response to divergent selection for ADG. The lines have undergone 10 generations of selection. Animals for the present experiments were sampled from F and S litters of the 10th generation in the first two consecutive sets of progeny from selected parents (fall 1993 and spring 1994). The establishment of the selection lines, selection procedures and methods used to sample the lines for present experiment were described in detail in Chapter III.

Experimental Procedures

The same barrows as in experiments 1 and 2 (Chapter III) were used to determine efficacy of exogenous CCK-8 on satiety response in ad libitum fed F and S pigs. Animals continued to be fed for zero growth after experiments 1 and 2 (Chapter III) and allowed a 48 hr acclimation period for this experiment. Each pig within a line was assigned randomly to one of the 24 sequences possible for four CCK-8 dosages of 0 (saline), 60,

100 or 300 ng/kg/min. The first dose was administered on the morning after the 48 hr acclimation period and at least 2 d separated successive doses. Doses of CCK-8 were infused (1 ml/min) via the jugular catheter for 12 min using a Harvard Model 22 infusion pump. Each infusion began 2 min before pigs were allowed ad libitum access to feed for 2 hr.

Individual feed intakes were determined at 10, 20, 30, 60, 60, 90 and 120 min after introduction of feed. Feed intakes during saline infusion established the control meal size for each animal. Methods used to collect individual feed intake data were the same as described in Chapter III.

Blood samples were collected via jugular catheter at 30, 15, and 2 min before and 10, 20, 30, 60, 90 and 120 min after introduction of feed. Blood samples (10 ml) were immediately transferred into glass tubes containing a 100 μl mixture of benzamidine (200 mg/ml) and heparin (500 IU/ml) that were held in an ice-water bath. Chilled blood samples were centrifuged for 10 min (1,500 × g) at 4 °C and recovered plasma samples were stored at –20 °C until assayed. Thirteen barrows from F and nine from S of the fall 1993 and spring 1994 farrowing groups completed the present study.

The details for plasma extraction and radioimmnoassay (RIA) procedures as well as the validation of the CCK-8 RIA were described in Chapter III. To evaluate CCK-8 concentrations within the range of standard curve, reconstituted plasma samples of some time intervals were diluted by assay buffer before RIA. For the 60 ng/kg/min dosage, reconstituted plasma samples of 10 and 20 min after feeding were diluted to 1 : 5 and 1 : 2, respectively. For the 100 ng/kg/min dosage, reconstituted plasma samples of 10, 20 and 30 min after feeding were diluted to 1 : 10, 1 : 5 and 1 : 2, respectively. For the 300

ng/kg/min dosage, the ratios of dilution for samples of 10, 20 and 30 min were 1 : 20, 1 : 10 and 1 : 5, respectively.

Calculations and Statistical Analysis:

Methods of least-squares analysis used for data from the present experiment were as follows. Plasma concentrations of CCK-8 at each of the interval time relative to introduction of feed and cumulative feed intake at 10, 20, 30, 60, 90 and 120 min during the feeding period were analyzed with a model that included the effects of farrowing group (fall 1993 or spring 1994), line, dosage, and all associated interactions, animal within farrowing group × line × dosage, time and all interactions of time with line, dosage and farrowing group. Effects of farrowing group, line, dosage and two- and three-way interactions of farrowing group, line and dosage were tested using animal within farrowing group × line × dosage as the error term. Effects of time and associated interactions were tested using the residual as the error term.

In addition to analysis of individual CCK-8 concentrations, areas under the curve for CCK-8 concentrations were calculated for each animal in the experiment by trapezoid method. Basal CCK-8 was based on the average of the three concentrations at –30, –15 and –2 min. Response area in CCK-8 was the difference between total area under the curve and the basal area. Values for basal CCK-8, response in CCK-8 and total CCK-8 were analyzed with a model that included the effects of farrowing group, line, dosage and all interactions.

Maximum decrement in intake occurred at 10 to 20 min after infusing 60, 100, and 300 ng/kg CCK-8. Intakes at 10 and 20 min after feeding were averaged for each dose of CCK-8 and the average values were used in data analysis. Average intakes at 10-

20 min after 60, 100 and 300 ng/kg CCK-8 infusion were expressed as percent of corresponding control intake after 0 ng/kg CCK-8 infusion. The relationship between percent of control intake and log dose of CCK-8 were analyzed by polynomial regression analysis (Fig.P, BioSoft, Durham, NC, USA). Comparisons of regression residual sum-of-squares and coefficient of determination determined that a first degree polynomial best described the dose-response effect of CCK-8 on acute intake in F and S pigs. Doses of CCK-8 inhibiting intake by 25% (D_{25}) and 50% (D_{50}) were calculated using the equations derived from regression analysis of the mean data in each group of pigs. Significance of difference in the mean D_{25} and D_{50} doses were determined using the 95% confidence interval range for each estimate.

Results

For the control groups (saline infusion), least-squares means for CCK-8 concentrations in plasma and for cumulative intakes are both presented in Figure 1. CCK-8 concentrations in plasma increased in response to feeding and saline in both F and S, but no significant differences were observed between the lines ($P > 0.40$). Intake after the control infusion of saline was greater ($P < 0.01$) in F than S throughout the 2 hr feeding period.

For the infusion groups, least-squares means for CCK-8 concentrations in plasma and for cumulative feed intake are plotted in Figure 2 to 4. CCK-8 concentrations in plasma were significantly affected by the infusion doses ($P < 0.01$) and time of feeding ($P < 0.01$) as well as by the dosage × time interaction ($P < 0.01$). Differences in plasma

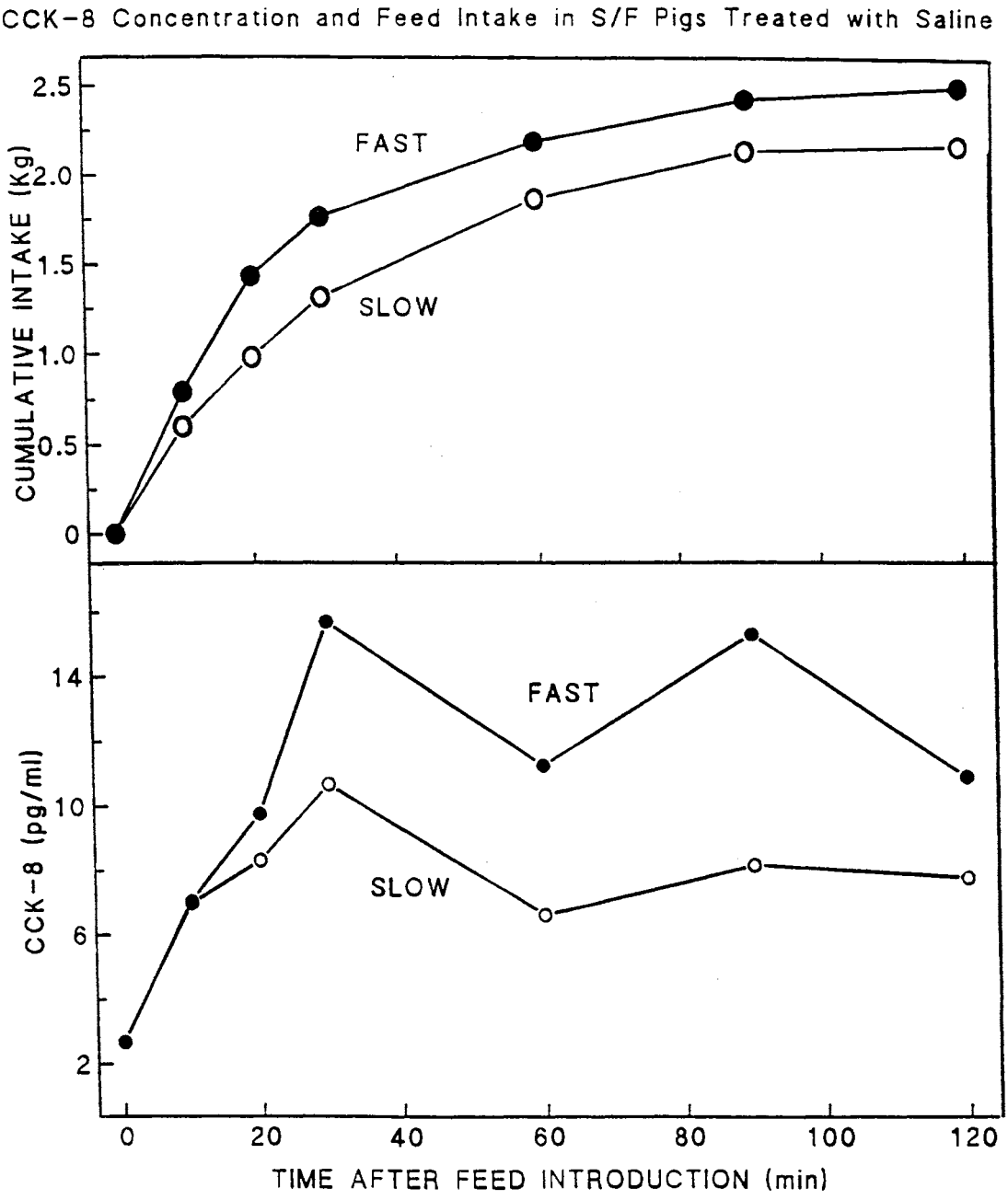

Figure 1. Mean cumulative feed intakes and CCK-8 concentrations in plasma in F and S treated with saline and allowed ad libitum intake for 2 hr. Pigs were fasted for hr before the introduction of feed at time 0.

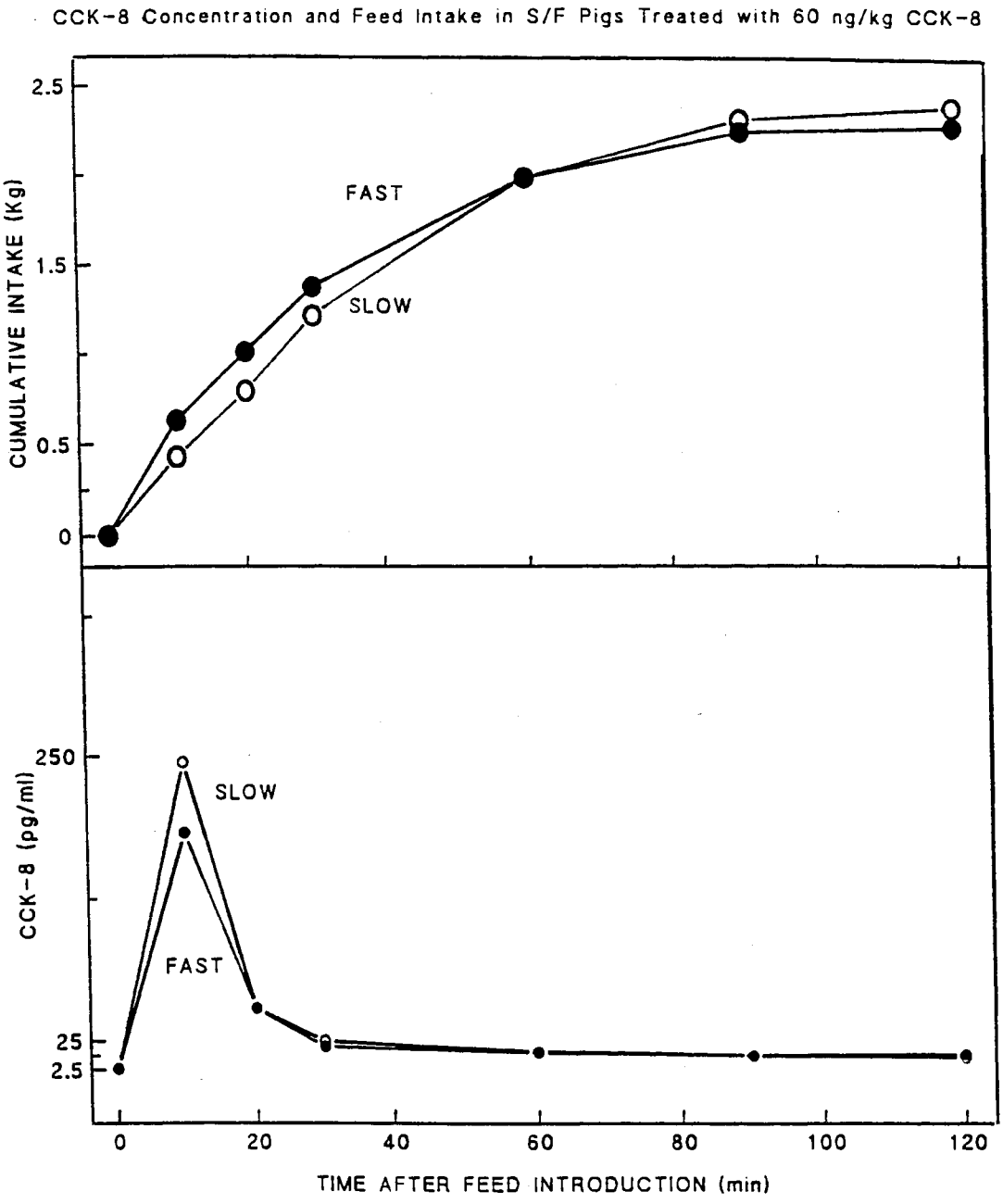

Figure 2. Cumulative feed intakes and CCK-8 concentrations in plasma from F and S pigs treated with 60 ng/kg of CCK-8 and fed ad libitum. Note the log scale on Y-axis for plasma CCK-8.

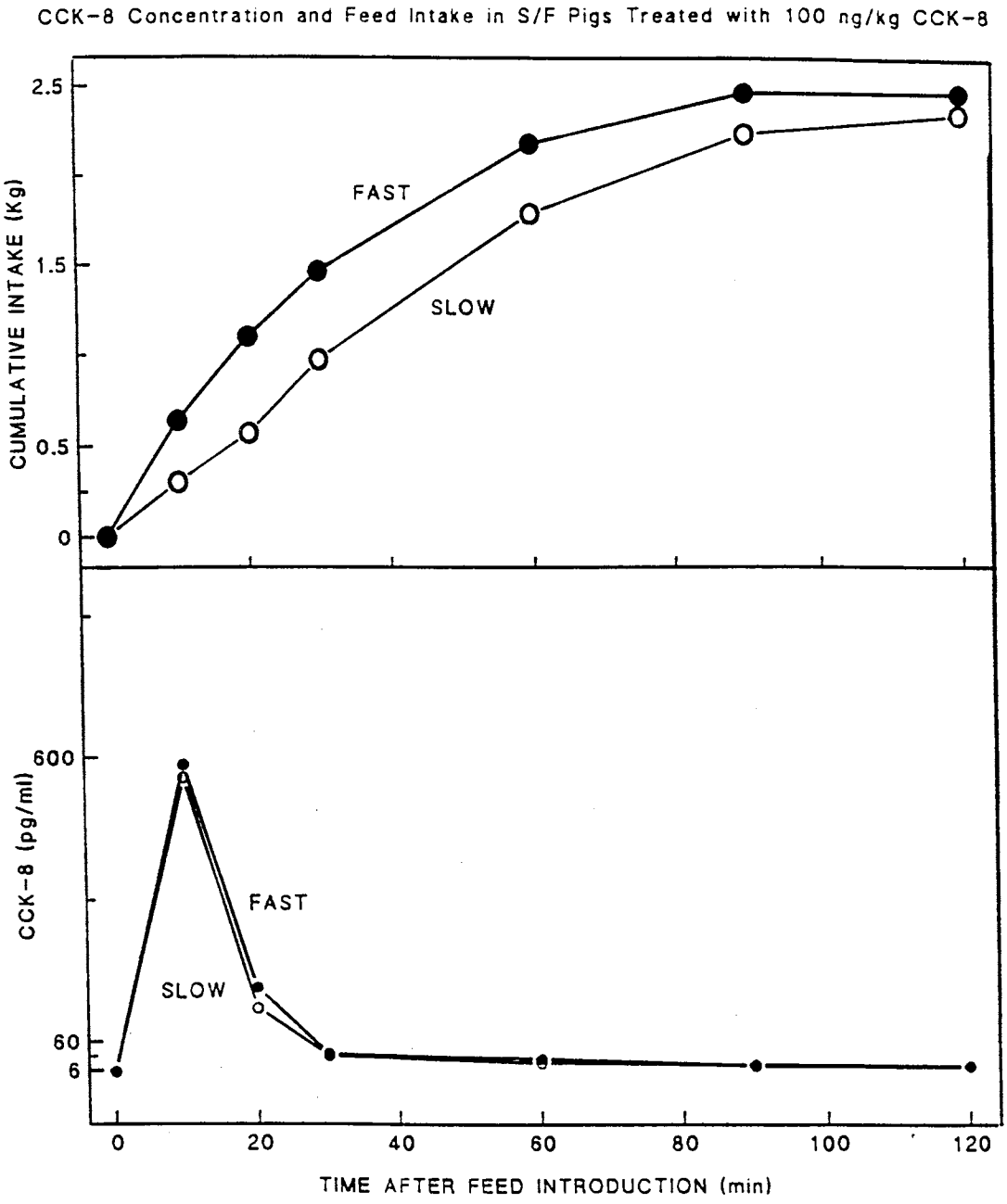

Figure 3. Cumulative feed intakes and CCK-8 concentrations in plasma from F and S pigs treated with 100 ng/kg of CCK-8 and fed ad libitum. Note the log scale on Y-axis for plasma CCK-8.

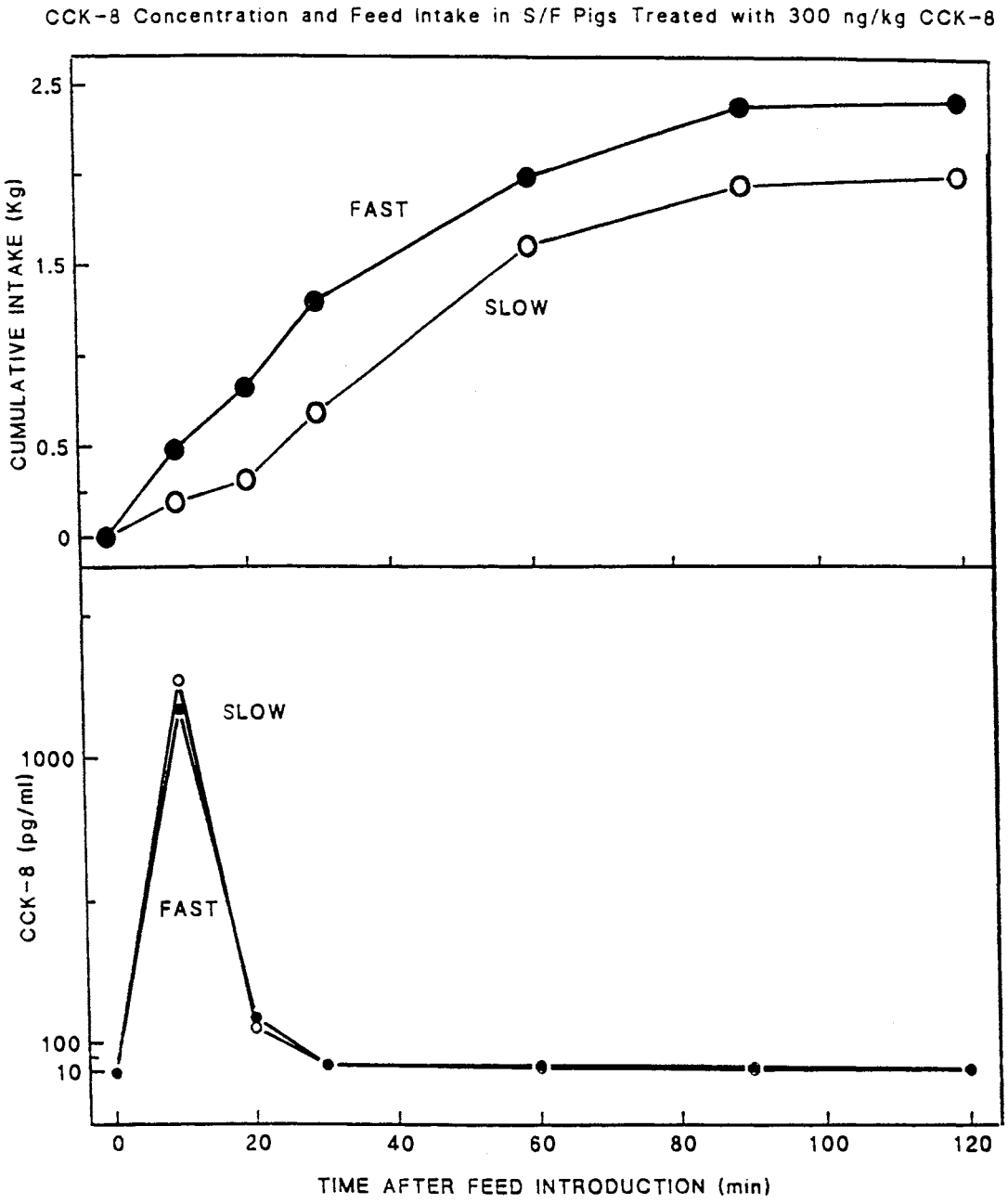

Figure 4. Cumulative feed intakes and CCK-8 concentrations in plasma from F and S pigs treated with 300 ng/kg of CCK-8 and fed ad libitum. Note the log scale on Y-axis for plasma CCK-8.

Table 1. LEAST-SQUARES MEANS FOR BASAL PLASMA CONCENTRATIONS OF CCK-8, RESPONSE AREAS IN CCK-8 AND TOTAL CCK-8 CALCULATED FROM THE AREA UNDER EACH CURVE[a].

	CCK-8 (pg/ml · min)		
	Basal	Response	Total
0 ng/kg/min[b]			
F	317.18 ± 81.98[c]	1108.07 ± 334.37	1425.24 ± 376.99
S	321.20 ± 85.02	618.32 ± 138.48	939.52 ± 170.85
60 ng/kg/min			
F	331.86 ± 38.82	3607.07 ± 479.46	3938.94 ± 488.56
S	434.80 ± 64.63	4369.98 ± 431.44	4554.78 ± 392.32
100 ng/kg/min			
F	429.09 ± 61.77	9255.32 ± 1321.75	9684.41 ± 1277.30
S	383.90 ± 61.44	8479.10 ± 564.68	8844.20 ± 534.57
300 ng/kg/min			
F	456.48 ± 77.78	15419.35 ± 2003.47	15875.95 ± 2011.08
S	453.00 ± 61.22	15379.58 ± 1271.53	15832.58 ± 1244.01

[a]Trapezoid method.

[b]Dosage for infusion of exogenous CCK-8.

[c]Least-square mean ± SE.

CCK-8 concentrations between lines were not detected ($P = 0.40$). Results of the analysis of basal CCK-8 and feeding-induced responses in CCK-8 are presented in Table 1. No significant differences in basal CCK-8 and response in CCK-8 detected between the lines ($P = 0.12$).

To allow comparison of the effects of CCK-8 in F and S pigs, cumulative intakes during the experimental period were recalculated as percentage of corresponding control intakes at each time point (Figure 5). Significant dose-dependent and time-dependent inhibitory effects of CCK-8 on intake were observed in both F and S line ($P < 0.01$). There were dosage × time and dosage × line interactions. Response to each dosage, averaged across the 2 hr feeding period, is shown in Figure 6. Increasing dosage of CCK-8 decreased intake in both lines, but by a significant greater amount in the S than in the F line ($P < 0.01$).

Maximum inhibition of intakes occurred during the first 20 min after infusion of any of the three doses of CCK-8 in both F and S (Figure 5). The dose-response curves of CCK-8 effect on feed intake are presented in Figure 7. The significant ($P < 0.05$) best fit regression line in F was $Y = -27.92$ (log dose) + 130.20 and that in S pigs was $Y = -60.57$ (log dose) + 181.18. CCK-8 dosages suppressing mean intake by 25% (D_{25}) and 50% (D_{50}) were calculated from these equations and were less ($P < 0.05$) in S (D_{25} = 56.6 ng/kg; D_{50} = 146.5 ng/kg) than in F (D_{25} = 94 7 ng/kg; D_{50} = 745 ng/kg) pigs (Figure 8). The results indicate a greater sensitivity in S than in F pigs to CCK-8.

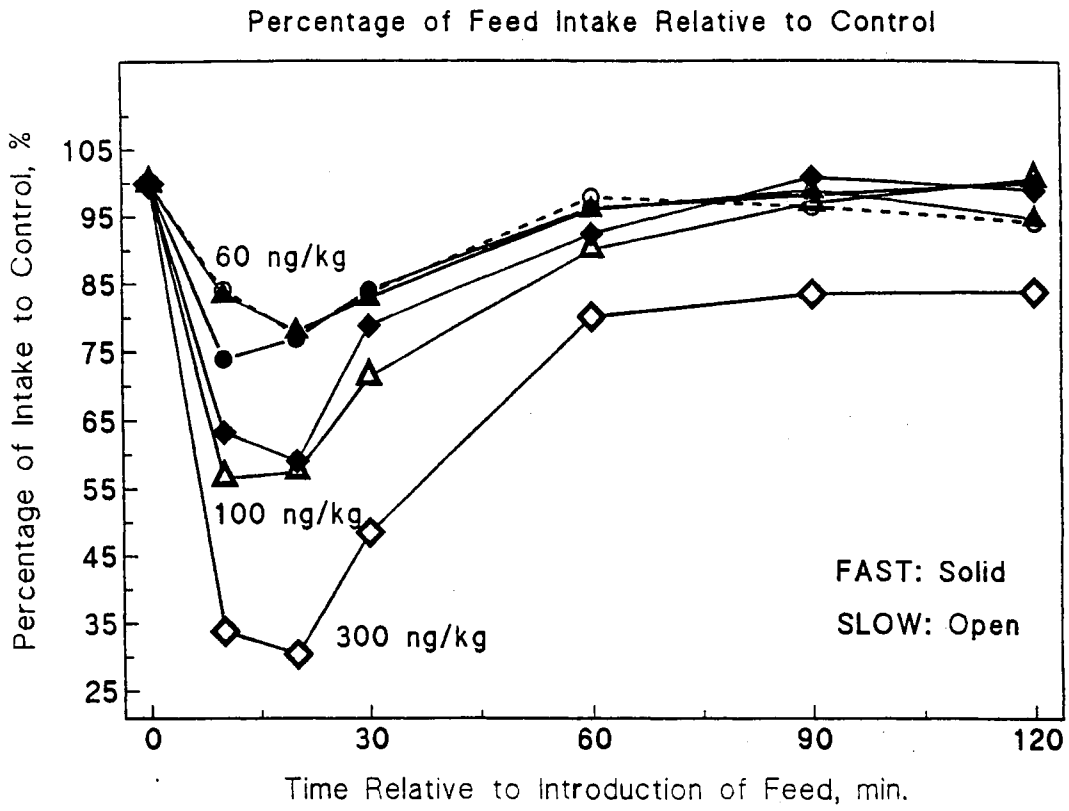

Figure 5. Mean percent cumulative intakes during the 2 hr of ad libitum feeding in F and S pigs treated with CCK-8. Intakes at each time point expressed as percent of corresponding intake after 0 ng/kg CCK-8.

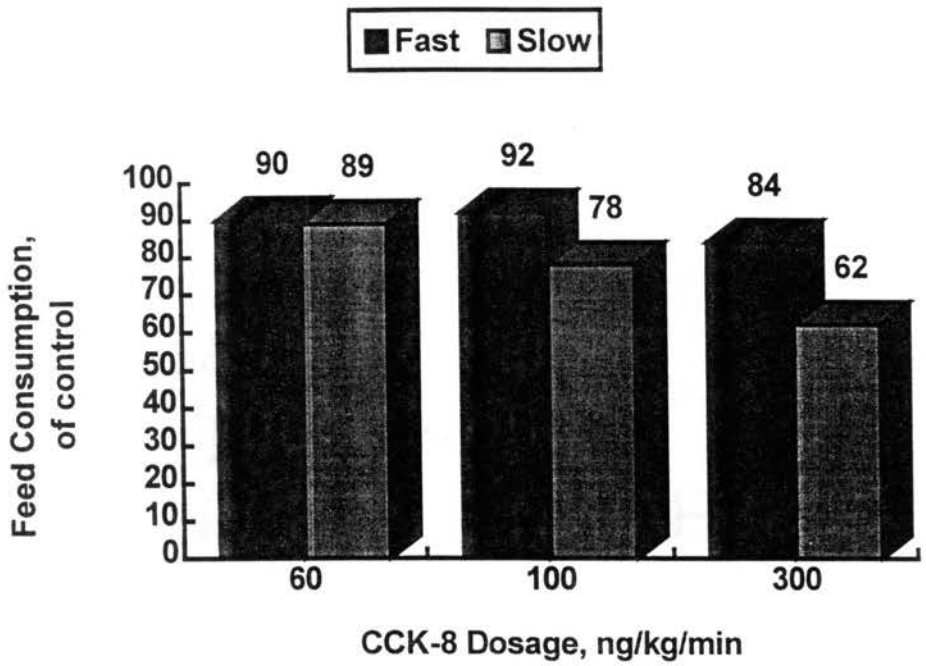

Figure 6. Percent of intake to control response to each dosage, averaged across the 2 hr feeding period.

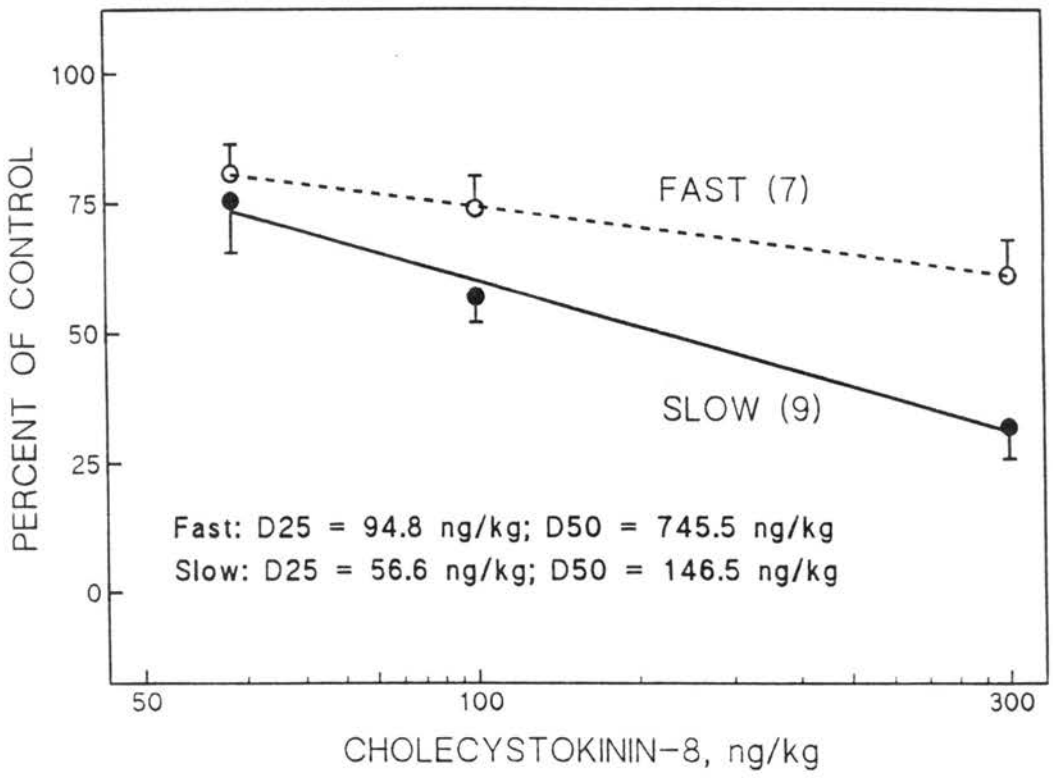

Figure 7. Dose-response effect of CCK-8 on acute (20 min) feed intake in F and S pigs.

Discussion

Results reported in Chapter III revealed that the cumulative feed consumption of barrows from F was greater than from S and CCK-8 concentrations in plasma in S tended to be higher than in F barrows. These results suggested CCK satiety action may play a role in genetic differences for appetite between the selection lines. The results from the present study again confirmed that the divergent selection for ADG may have targeted genes involved CCK satiety action pathways.

In the present studies, significant dose-dependent and time-dependent inhibitory effects of CCK-8 on intake were observed in F and S (Figure 7). These results are in agreement with previous reports in pigs (Anika et al., 1981; Houpt et al., 1979, Baldwin et al., 1982).

Feed consumption was reduced in both lines as dosage of CCK-8 increased, but the reduction was greater in S than in F. Maximum inhibition of intakes occurred during the first 20 min after infusion of either of the three doses of CCK-8 in both lines. These results are consistent with the suggestion that CCK-8 acts as an acute, short term satiety signal (Reidelberger, 1994; Morley, 1995). Calculated dose of CCK-8 suppressing mean intake by 25% or 50% was less in S than in F pigs. This dose range was in agreement with another report in which 67 ng/kg CCK-8 infused into the jugular vein in pigs suppressed intake by 35% (Houpt et al., 1979).

The present results indicated a greater sensitivity in S than in F pigs to CCK-8. The relatively greater inhibitory effects of CCK-8 in S than F pigs were associated with similar plasma concentrations and patterns of CCK-8 in both S and F after infusion of

either 60, 100 or 300 ng/kg dose of CCK-8. Similar results were reported in obese men (Kissileff et al., 1981), obese mice (Strohmayer et al., 1981; MaLaughlin et al., 1981) and obese Zucker rats (McLaughlin et al., 1980) in which obese individuals expressed a decrease in sensitivity to satiety-inducing effects of CCK-8. Thus, decreased sensitivity to CCK, which appears to be exhibited by obese animals, may be a cause or consequence of obesity and increased meal size (Baile et al., 1986).

Taken together, results from the present experiments indicate that the genetic difference between these lines for appetite may be partly due to a difference in sensitivity to this satiety signal. Metabolic clearance of the infused CCK-8 may be similar in both lines because of the similar concentrations and patterns of plasma CCK-8 in S and F pigs. The differences in sensitivity to satiety action of CCK may be caused by differences in DNA frequencies encoded in the genes for CCK receptors (CCKAR or CCKBR) involved with regulation of appetite. To obtain a more complete picture of the CCK action in genetic variation between F and S on protein levels, additional experiments to determine effects of specific CCK receptor antagonists, CCK immunoneutralization, distribution patterns and molecular forms of CCK (HPLC/RIA) on feed intake in F and S are necessary.

Direct and correlated responses to selection are due to changes in the frequencies of gene alleles affecting each of the measured traits (Falconer, 1989). In pigs, genetic correlation of ADG with daily feed intake is quite high (Buchanan et al., 1993). Selection for ADG, and indirectly for feed intake, has no doubt targeted the alleles of many genes involving in feeding control pathway. Results from Chapter III and from the present

experiment suggest that the CCK gene and genes encoding CCK receptors may be targeted by the selection for ADG in pigs.

Current approaches for genome analysis in porcine (Archibald et al., 1995; Rohrer et al., 1995) provide opportunity to identify the molecular basis of genetic variation in growth, feed intake and efficiency. To detect important loci that influence traits of interest, the first step is to establish a resource family in which the relevant alleles are segregating at those loci (Lander et al., 1989). The phenotypic and physiological divergence detected between the F and S lines in the present and previous studies (Woltmann et al., 1992, 1995; Clutter et al., 1995) suggest that the F and S lines can be used to produce a resource family to search for loci contributing to variation in feed intake and growth.

In summary, results from the present experiments reveal a greater sensitivity to CCK-8 in S than in F in suppressing feed intake and support the hypothesis that the line selected for fast growth has lesser sensitivity to the satiety effects of CCK-8. At the DNA level, further studies are needed to determine the relative contributions of the CCK gene and CCK receptor genes to variation in appetite in resource families in which alleles of these genes are segregating.

CHAPTER V

RELATIONSHIP OF CHOLECYSTOKININ GENOTYPES WITH GROWTH AND BACKFAT IN OFFSPRING OF SIRES PRODUCED BY THE CROSS OF GENETICALLY DIVERGENT LINES

Abstract

The objective of this study was to identify relationships of a marker for the CCK gene with postweaning average daily gain (ADG) and 10th rib backfat thickness (BF) in a population of swine. Two families were produced using F_1 sires obtained from the cross of lines divergently selected for either fast or slow ADG. Two F_1 sires (A and B) were each mated to 15 unrelated dams and produced 147 and 132 offspring, respectively, in the resulting half-sib families. Alleles of the marker for the CCK gene located on chromosome 13 were significantly associated with ADG in the family of sire A ($P < 0.02$), but not in the family of sire B. There was a tendency for relationship between alleles of the CCK marker and BF in the family of sire A ($P < 0.1$), but not in the family of sire B. Our results suggested that there may be a QTL for ADG on chromosome 13. Further analyses of the family of sire A, using additional markers in the region of the CCK gene are necessary to verify that a QTL is present and, if so, to more closely determine its position.

Introduction

Swine breeders have used traditional methods (e.g., phenotypic selection) to make considerable genetic advancement in some performance traits. Genetic improvement of production efficiency may be enhanced by molecular marker-assisted selection (MAS) (Soller, 1994; Weller, 1996). Molecular marker-assisted selection will first require identification of candidate genes or anonymous genetic markers associated with important traits. Only a few studies have shown associations of genes or anonymous markers with quantitative trait loci (QTL) in pigs (e.g., Jung et al., 1989; Clamp et al., 1992; Andersson et al., 1994; Yu et al., 1995; Rothschild et al., 1995). Studies designed to determine the association of specific genes with genetic variation in traits of interest (i.e., "the candidate gene approach") are justified when the genes are known to have functions related to characteristics of production or reproduction.

Feed efficiency is one of the major factors that determine the profitability of the swine industry, but traditional methods of genetic improvement in feeding efficiency have be hampered by the cost of measuring individual feed intake. A better understanding of the genetic control of appetite regulation on DNA level may lead to the development of more effective selection methods (e.g., MAS).

The hormone CCK has been reported to play a role in appetite regulation in various species including pigs (Anika et al., 1981; Della-Fera et al., 1979; McLaughlin et al.,1985). Specific antagonists for CCK receptors (Ebenezer et al., 1990; Baldwin et al., 1992), and specific immunoneutralization of circulating CCK-8 (Pekas et al., 1993), have resulted in increased feed consumption in pigs. Our previous studies of physiological

divergence of fast (F) or slow (S) growth selection lines of pigs revealed a tendency for greater plasma concentrations of CCK-8 (Chapter III) and a greater sensitivity in response to exogenous CCK-8 in S than in F barrows (Chapter IV), both results consistent with the relative feed consumptions of the lines. Thus, CCK action may play a role in the genetic differences in appetite between F and S. However, these physiological evaluations of selection lines only provide a picture of the genetic differences at the protein level. The relative contributions of the CCK gene to variation in appetite and growth can only be determined in families in which markers for the CCK gene are segregating. The phenotypic and physiological divergence detected between the F and S lines in the present and previous studies (Woltmann et al., 1992, 1995; Clutter et al., 1995) suggests the lines as candidates to produce a resource family in which loci contributing to variation in feed intake and growth can be identified.

The objective of the present study was to examine the contribution of chromosomal regions linked to the CCK gene to variation in growth and body composition in the offspring of F_1 sires produced by the cross of the F and S selection lines.

Materials and Methods

Resource Families

Half-sib families were produced by mating two F_1 sires (A and B) each with 15 unrelated sows at the University of Wisconsin-Platteville Swine Center. Both sires were obtained from the cross of lines divergently selected for either fast or slow ADG for 10

generations (Woltmann et al., 1992; Clutter et al., 1995). A total of 147 and 132 offspring, respectively, were produced in the half-sib families.

Phenotypic Traits

Offspring were evaluated for ADG, measured as the difference in live weight before slaughter (approximately 110 kg) and at weaning, divided by days from weaning to slaughter. This was also the sole selection criterion in the F and S lines (Woltmann et al., 1992). BF at the 10th rib was measured on cold carcasses 24 hr after slaughter, as an indicator of body composition.

DNA analysis

DNA samples in the study were obtained from a collaborative laboratory at University of Wisconsin-Madison. Working dilutions of extracted DNA were obtained for each individual at a concentration of 50 ng/μl. Methods of animal handling, DNA extraction and preparation have been described by Casas-Carrillo et al. (1997ab).

The marker for the CCK gene was previously described by Clutter et al. (1996). Briefly, the primers used to amplify the porcine CCK gene were designed on the basis of the exon/intron organization of the human and mouse CCK genes (Takahashi et al., 1985, 1986; Vitale et al., 1991) and porcine cDNA sequences (Gubler et al., 1984) and were to flank an expected intron of ~4 to 6 kb. Primer sequences were:

5'-primer, 5'-CTGGCCAGATACATCCAGCA-3';

3'-primer, 5'-ATCCATCCAGCCCATGTAGT-3'.

Polymerase chain reaction (PCR) [25 μl final volume] was performed using 50 ng genomic DNA, 1 mM $Mg(OAc)_2$, 200 μM each dNTP, 0.35 μM each primer, 0.4 units rTth DNA polymerase-XL (Perkin-Elmer) and its supplied buffer. A hot start was used

(polymerase added at 80 °C), and thermal cycling began with an initial cycle of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 5 min, followed by 34 cycles of 94 °C for 30 sec, 60 °C for 1 min and 72 °C for 5 min. The last 19 cycles included additional, cumulative extension periods of 15 sec per cycle. The reaction resulted in a single DNA product of ~3.57 kb. Terminal end-sequencing was used to verify that the product includes the expected exon regions of the porcine CCK gene. Digestion of the PCR product by the restriction enzyme *Dpn*II produced a restriction fragment length polymorphism (RFLP) that could be used as a marker for the CCK gene. The genotypes of individuals were expressed as AA, AB and BB. Both sires (A and B) were heterozygous (AB) for the marker, which allowed the analysis of relationships between the CCK marker and phenotypic traits in each of the half-sib families.

Statistical analysis

Inheritance of paternal alleles for CCK was coded as the probability of inheriting allele A from the sire (Dentine et al., 1990). Where paternal inheritance was unambiguous, this probability was either 1 or 0. In cases where the offspring was ambiguous, that is, their heterozygous genotype was identical to that of sire and dam, the probability of inheriting sire allele A was 0.5. These probabilities were used as regressors in the statistical model. Data for ADG and BF were analyzed separately for each sire group with least-squares procedures using the following model:

$$Y_{ij} = \mu + L_i + \beta_{marker}X_{marker} + \varepsilon_{ij}$$

where:

Y_{ij} = j-th observation from the i-th litter,

μ = population mean,

L_i = effect of the i-th litter,

β_{marker} = regression on probability of inheriting marker allele A from sire,

X_{marker} = probability of inheriting marker allele A from sire,

ε_{ij} = random error.

Results

PCR products were obtained for 60 of 147 individuals in the family of sire A and 36 of 132 individuals in the family of sire B. Due to missing phenotypic measurements, only 46 and 25 observations could be used in the analyses for the families of sire A and of sire B, respectively.

There was a significant association between the inheritance of paternal marker alleles and ADG in the family of sire A ($b = 0.032 \pm 0.014$ kg/d, $p < 0.02$). For the family of sire B, CCK genotypes were not associated significantly with ADG ($b = 0.014 \pm 0.022$ kg/d, $p > 0.60$).

CCK marker genotypes tended to be associated with 10th rib BF in the family of sire A ($b = 0.52 \pm 0.31$ cm, $p < 0.1$), but not in the family of sire B ($b = 0.13 \pm 0.51$ cm, $p > 0.80$).

Discussion

The candidate gene approach is justified when genes previously identified in the species of interest or other species have functions related to the traits of interest. Several

studies have revealed associations of genes with growth traits in mice (Winkelman et al., 1992; Pomp et al., 1994; Horvat et al., 1995; Keightley et al., 1996). In pigs, only a few studies have shown the associations of genes with quantitative traits.

Clamp et al. (1992) reported the presence of gene(s) that affect rate of body weight gain linked to the glucose phosphate isomerase (GPI) loci on porcine chromosome 6. In a study for detecting the relationships of growth hormone and insulin-like factor-1 genotypes with growth and carcass traits in pig families, a potential association of IGF1 genotype with ADG was observed and a QTL, closely linked to IGF1, may be located in the interval between Sw1071 and IGF1 on chromosome 5 (Casas-Carrillo et al., 1997a). Significant associations of PIT1 genotypes with growth rate were detected; thus, PIT1 has been suggested to be a candidate gene for a QTL for growth on chromosome 13 (Yu et al., 1995).

CCK has been established as a satiety hormone in the pig. However, the relative contribution of the CCK satiety action to variation in appetite and growth is still unknown. This could be verified by analyzing the associations between marker genotypes for CCK and CCK receptors with growth and feed intake traits in resource families in which there is segregation at loci contributing to variation in growth and feed intake. Due to the lack of phenotypic measurements for feed intake, only the relationship of CCK genotype with ADG and BF was evaluated in the present study.

The CCK gene was reported to be located on porcine chromosome 13 (Clutter et al., 1996; Rettenberger et al., 1996). A briefly linkage map for chromosome 13 is presented in Figure 1. The CCK gene marker used in the study had no recombination with the Type I marker ITIH, was linked to the marker TF with a recombination

frequency of 0.19, and to microsatellite marker S0288 with recombination frequency of 0.02 (Clutter et al., 1996). The frequencies of alleles of the marker for the CCK gene were not significant different between F and S (Clutter et al., 1996).

A nominally significant association between the marker alleles and ADG and a tendency for association between the marker alleles and BF were observed in the family of sire A in present study. The results indicate that a potential QTL for ADG may be segregating in the region of the CCK gene on chromosome 13. The results are consistent with other studies in which potential QTLs for growth have been reported to be located on the porcine chromosome 13 in different resource families (Yu et al., 1995; Andersson et al., 1994). In the same resource families as used in the present investigation, however, no significant association of ADG with the genomic region on chromosome 13 was detected by scanning the genome with genetic markers (Casas-Carrillo et al., 1997b). This result is in contrast with the present results. A limited number of markers used in their study may be one of the possible reasons for failing to detect the significant associations on chromosome 13.

Although a significant association was detected in one family, the power for association detection was limited by the small amount of data due to unsuccessful PCR amplification and missing phenotypic measurements. Unsuccessful PCR may be due to a break down of the genomic DNA. Thus, additional work is needed to evaluate smaller size markers such as microsatellite markers which are in the chromosome region of the CCK gene (e.g., S0288) and apply interval mapping analysis to confirm and refine the present results.

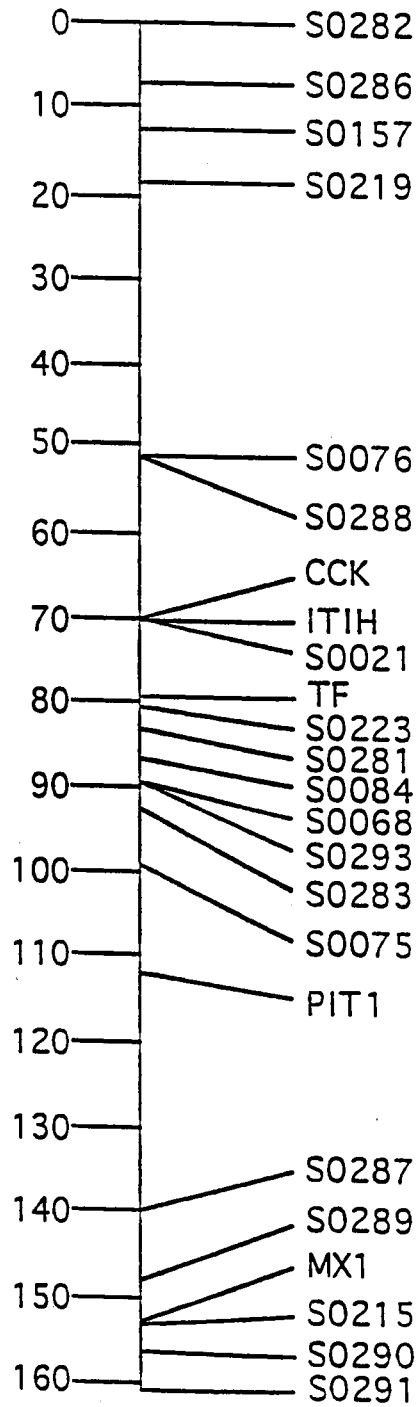

Figure 1. Linkage map of pig chromosome 13. Map distances (cM) between markers are presented. The location of a potential ADG QTL was suggested to be in the region linked to the CCK gene. (Derived from Pig Genome Mapping, Roslin Institute, UK)

CHAPTER VI

SUMMARY

Genetic variation in appetite exists in animal populations. Better understanding of the genetic nature of appetite regulation may help development of more effective selection methods and better breeding programs for feed efficiency. Many animal models have been established to identify the molecular mechanisms of control of feed intake, regulation of body weight and development of obesity. However, all of these animal models are based on single gene mutations. Studies of normal quantitative genetic variation in feed intake and growth are rare. To our knowledge, no research has been reported to clarify the quantitative and molecular basis for variation in appetite regulation in normal animal populations using divergent selection lines as a model.

In the present study, the selection lines of pigs with divergent potential in growth and feed intake were used to elucidate the role of satiety hormone CCK in appetite regulation. The genetic differences for appetite between the lines may be in part due to differences in satiety effects of CCK. Lesser satiety effects of CCK-8 in F than in S may be caused by a lower concentration of plasma CCK-8 in F or by changed/unchanged plasma concentrations of CCK-8 together with decreased cellular response (i.e., CCK-8 activation of CCK receptors involved with regulation of appetite). A tendency for greater

plasma concentration of CCK-8 and a significantly greater sensitivity to exogenous CCK-8 in S than in F pigs was revealed in the present study.

Although the importance of CCK-8 as a satiety signal in the pig has been implied through infusion studies, antagonists for CCK-8 and the immunoneutralization of circulating CCK-8, the relative effects of bioactive forms of CCK on feeding behavior remains to be determined. The effect of selection on relative changes of molecular forms of CCK in the present study can only be determined by additional work to separate peptide fractions (HPLC/RIA). In addition, experiments to determine the effects of CCK receptor antagonists and CCK immunoneutralization on feed intake in F and S may provide a more complete picture of the role of CCK action in genetic differences between the lines.

To understand genetic variation in growth and body composition at the DNA level, resource families were produced by using the cross of the F and S selection lines as F_1 sires. A significant association was detected between the variation in ADG and a marker for the CCK gene. If this association is real, the effect may be due to alleles of the CCK gene or of some closely linked gene(s).

Taken together, we concluded that the CCK satiety action plays a role in genetic variation in appetite in F and S and there may be a QTL for ADG in the chromosomal region of the CCK gene on chromosome 13.

However, due to the complex and quantitative characteristics of feed intake, many loci probably contribute to genetic variation in appetite. For example, the frequencies of alleles of markers for the leptin gene and for the CCKAR gene were significant different between F and S, indicating that these loci may have been targets of selection and

contribute to genetic difference between the lines. Also, another potential QTL for ADG was detected on chromosome 3 in the family of one F_1 sire. Thus, additional work is necessary to learn more about genetic variation in feed intake. A genome scanning approach will be continued to identify important chromosomal regions affecting feed intake and efficiency. Then, the identified regions with suspected QTL or ETL for feed intake will be investigated using comparative mapping and positional candidates to try to identify the individual genes responsible for differences in appetite. Furthermore, the expression and function of relevant candidate genes at the molecular and physiological levels will be examined in detail. This multidisciplinary approach of positional candidate cloning and gene expression/function research will help to elucidate how genes affect feed intake and efficiency and how they can be manipulated for genetic improvement.

BIBLIOGRAPHY

Akabayashi, A., Wahlestedt, C., Alexander, J. T. and Leibowitz, S. F. (1994) Specific inhibition of endogenous neuropeptide Y synthesis in arcuate nucleus by oligonucleotides suppresses feeding behavior and insulin secretion. Mol. Brain Res. 21: 55-61.

Alavi, F. K., McCann, J. P., Sangiah, S. and Mauromoustakos, A. (1991) Effects of naloxone on ad. libitum intake and plasma insulin, glucose, and free fatty acids in maintenance-fed sheep. Domes. Anim. Endocrinol. 8: 109-115.

Anand, B. and Brobeck, J. R. (1951) Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. 24: 123-146.

Andersson, L., Haley, C. S., Ellegren, H., Knott, S. A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I., Hakansson, J. and Lundstrom, K. (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263: 1771-1774.

Anika, S. M., Houpt, T. R. and Houpt, K. A. (1981) Cholecystokinin and satiety in pigs. Am. J. Physiol. 240: R310-R318.

Arbona, J. R., Marple, D. N., Russell, R. W., Rahe, C. H., Mulvaney, D. R. and Sartin, J. L. (1988) Secretory patterns and metabolic clearance rate of porcine growth hormone in swine selected for growth. J. Anim. Sci. 66: 3068-3072.

Arbona, J. R., Rahe, C. H., Kelley, R. L., Sinha, Y. N., Strength, D. R., Marple, D. N. and Mulvaney, D. R. (1992) Differences in GH secretion from individual somatotropes in rats genetically selected for fast and slow growth. Am. J. Physiol. 263: E748-E751.

Archibald, A. L., Haley, C. S., Brown, J. F., Couperwhite, S., McQueen, Htested . A., Nicholson, D., Coppieters, W., van de Weghe, A., Stratil, A., Winter∅, A. K., Fredholm, M., Larsen, N, J,, Nielsen, V. H., Milan, D., Woloszyn, N., Robic, A., Dalens, M., Riquet, J., Gellin, J., Caritez, J-C., Burgaud, G., Ollivier, L., Bidanel, J-P., Vaiman, M., Renard, C., Geldermann, H., Davoli R., Ruyter,, D., Verstege, E. J. M., Groenen, M. A. M., Davies, W., H∅yheim, B., Keiserud, A., Andersson, L., Ellegren, H., Johansson, M., Marklund, L., Miller, J. R., Anderson Dear, D. V., Signer, E., Jeffreys, A. J., Moran, C., LeTissier, P., Muladno, Rothschild, M. F., Tuggle, C. K., Vaske, D., Helm, J., Liu, H-C., Rahman, A., Yu, T-P., Larson, R. G. and Schmitz, C. B. (1995) The PiGMaP consortium linkage map of the pig *(Sus scrofa).* Mamm. Genome 6: 157-175.

Baber, N. S., Dourish, C. T. and Hill, D. R. (1989) The role of CCK, caerulein and CCK antagonists in nociception. Pain 39: 307-328.

Baile, C. A., Keim, D. A., Della-Fera, M. A. and McLaughlin, C. L. (1981) Opiate antagonists and agonists and feeding in sheep. Physiol. Behav. 26: 1019-1023.

Baile, C. A., Keim, D. A., Della-Fera, M. A. and McLaughlin, C. L. (1983) Hormones and feed intake. Proc. Nutr. Soc. 42: 113-127.

Baile, C. A., McLaughlin, C. L. and Della-Fera, M. A. (1986) Role of cholecystokinin and opioid peptides in control of food intake. Physiol. Rev. 66: 172-234.

Baird, D. M., Nalbandov, A. V. and Norton, H. W. (1952) Some physiological causes of genetically different rates of growth in swine. J. Anim. Sci. 11: 292-300.

Baldwin, B. A., Cooper, T. R. and Parrott, R. F. (1982) Effect of cholecystokinin octapeptide on food intake in pigs. Proc. Nutr. Soc. 41: 119-121.

Baldwin, B. A. and De La Riva, C. (1992) Effects of food intake in pigs of antagonists acting on cholecystokinin type A and B receptors. J. Physiol. 446: 291P.

Baldwin, B. A., De La Riva, C. and Gerskowitch, V. P. (1994) Effect of a novel CCK-A receptor antagonist (2-NAP) on the reduction in food intake produced by CCK in Pigs. Physiol. Behav. 55: 175-179.

Barton, C., York, D. A. and Bray, G. A. (1996) Opioid receptor subtype control of galanin-induced feeding. Peptides 17: 237-240.

Bechara, A. and van der Kooy, D. (1985) Opposite motivational effects of endogenous opioids in brain and periphery. Nature 314: 533-534.

Beck, B., Burlet, A., Niclas, J. P. and Burlet, C. (1990) Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: implications in feeding and sexual behaviors. Physiol. Behav. 47: 449-453.

Beinfeld, M. C., Lewis, M. E., Eiden, L. E., NiLaver, G. and Pert, C. B. (1983) The distribution of cholecystokinin in rhesus monkey brain as determined by radioimmunoassay. Neuropeptides 3: 337-344.

Beinfeld, M. C., Meyer, D. K., Eskay, R. L., Jensen, R. T. and Brownstein, M. J. (1981) Distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. Brain Res. 212: 51-57.

Billington, C. J., Briggs, J. E., Brace, M. and Levine, A. S. (1991) Effects of intracerebroventicular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. 206: R321-R327.

Billington, C. J., Herman, B. H., Bartness, T. J., Levine, A. S. And Morley, J. E. (1990) Effects of the opiate antogonists diprenorphine and naloxone and selected opiate agonists on feeding behavior in guinea pigs. Life Sci. 46: 147-154.

Bitar, K. N. and Makhlouf, G. M. (1982) Receptors on smooth muscle cells: characterization by contraction and specific antagonists. Am. J. Physiol. 242: G400-G407.

Blanke, S. E., Johnsen, A. H. and Rehfeld, J. F. (1993) N-terminal fragments of intestinal cholecystokinin---evidence for release of CCK-8 by cleavage on the carboxyl side of arg(74) of proCCK. Regul. Pept. 46: 575-582.

Braden, N., Merand, Y., Rouleau, D., Moore, S., Dockray, G. J. and Dupont, A. (1981) Regional distributions of somatostatin and cholecystokinin-like immunoreactivities in rat and bovine brain. Peptides 2: 299-302.

Brand, S. J. and Morgan, G. H. (1981) The release of rat intestinal cholecystokinin after oral trypsin inhibitor measured by bioassay. J. Physiol. Lond. 319: 325-343.

Bray, G. A., Shimizu, H., Retzios, A. D., Shargill, N. S. and York, D. A. (1988) Reduced acetylation of melanocyte stimulating hormone(MSH): a biochemical explanation for the yellow obese mouse. In: Obesity in European 88. pp. 259-270. Edited by P. Bjorntorp and S. Rossner. John Libbey, London.

Bray, G. A. and York, D. A. (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59: 719-809.

Brief, D. J. and Davis, J. D. (1994) Reduction of food intake and body weight by chronic intraventricular insulin infusion. Brain Res. Bull. 12: 571-575.

Buchanan, D. S., Clutter, A. C., Northcutt, S. L. and Pomp, D. (1993) Animal breeding: principles and applications. 4th edition. Oklahoma State University.

Bultman, S. J., Michaud, E. J. and Woychich, R. P. (1992) Molecular characterization of the mouse *agouti* locus. Cell 71: 1195-1205.

Bultman, S. J., Russell, L. B., Gutierrez-Espeleta, G. A. and Woychick. R. P. (1991) Molecular characterization of a region of DNA associated with mutations at the *agouti* locus in the mouse. Proc. Natl. Acad. Sci. USA 88: 8062-8066.

Calam, J., Ellis, A. and Dockray, G. J. (1982) Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. J. Clin. Invest. 69: 218-225.

Campfield, L. A., Smith, F. J., Guisez, Y., Devo, R. and Burn, P. (1995) Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269: 546-549.

Cantor, P. (1986) Evaluation of radioimmunoassay for cholecystokinin in human plasma. Scand. J. Clin. Invest. 46: 213-221.

Cantor, P. (1989) Cholecystokinin in plasma. Digestion 42: 181-201.

Cantor, P. and Rehfeld, J. F. (1987) The molecular nature of cholecystokinin in human plasma. Clin. Chim. Acta. 168: 153-158.

Cantor, P. and Rehfeld, J. F. (1989) Cholecystokinin(CCK) in pig plasma: release of components devoid of a bioactive CCOH-terminus. Am. J. Physiol. 256: G53-G61.

Casas-Carrillo, E., Prill-Adam, A., Price, S. G., Clutter, A. C. and Kirkpatrick, B. W. (1997a) Relationship of growth hormone and insulin-like growth hormone genotypes with growth and carcass traits in offspring of purebred and crossbred swine sires. Anim. Genet. (in press).

Casas-Carrillo, E., Prill-Adam, A., Price, S. G., Clutter, A. C. and Kirkpatrick, B. W. (1997b) Mapping genomic regions associated with growth rate in offspring of swine sires produced by the cross of genetically divergent lines. J. Anim. Sci. (in press).

Chang, T. M. and Chey, W. Y. (1983) Radioimmunoassay of cholecystokinin. Dig. Dis. Sci. 28: 456-469.

Chen, H., Charlat, O., Tartaglia, L. A., Woolf., Weng, X., Ellis, S. J., Lakey, N. D., Clulpepper, J., Moore, K. J., Breitbart, R. E., Duyk, G. M., Tepper, R. I. and Morgenstern, J. P. (1996) Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the receptor gene in *db/db* mice. Cell 84: 491-495.

Chua Jr, S. C., Chung, W. K., Wu-Peng, X. S., Zhang, Y., Lui, S. M., Tartaglia, L. and Leibel, R. L. (1996) Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271: 994-996.

Clamp, P. A., Beever, J. E., Fernabde, R. L., MaLaren, D. G. and Schook, L. B. (1992) Detection of linkage between genetic markers and genes that affect growth and carcass traits in pigs. J. Anim. Sci. 70: 2695-2706.

Clark, J. T., Kalra, P. S., Crowley, W. R. and Kalra, S. P. (1984) Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 115: 427-429.

Clutter, A. C. (1992) Selection for growth-experimental results. Record of Proceedings National Swine Improvement Federation Conference and Anuual Meeting.

Clutter, A. C., Jiang, R., McCann, J. P. and Buchanan, D. S. (1997) Plasma cholecystokinin-8 in pigs with divergent genetic potential for feed intake and growth. Domest. Anim. Endocrinol. (submitted).

Clutter, A. C., Sasaki, S. and Pomp, D. (1996) PCR-based polymorphisms in the porcine *cholecystokinin* (CCK) gene and assignment to chromosome 13. Anim. Genet. 27: 369-370.

Clutter, A. C., Spicer, L. J., Woltmann, M. D., Grimes, R. W., Hammond, J. M. and Buchanan, D. S. (1995) Plasma growth hormone, insulin-like growth factor I, and insulin-like growth factor binding proteins in pigs with divergent genetic merit for postweaning average daily gain. J. Anim. Sci. 73: 1776-1783.

Clutter, A. C., Pomp, D. and Sasaki, S. (1996) Development of polymorphic markers within candidate loci for appetite regulation and estimation of allelic frequencies in lines of pigs with divergent genetic potential for feed intake. XXVth Internatl. Conf. Anim. Genet. p. 178.

Coleman, D. L. (1978) Obesity and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14: 141-148.

Corp, E., McQuade, S., Moran, T. H. and Smith, G. P. (1993) Characterization of type A and type B CCK receptor binding sites in rat vagus nerve. Brain Res. 623: 161-166.

Covasa, M. and Forbes, J. M. (1994) Exogenous cholecystokinin octapeptide in broiler chickens: satiety, conditioned color aversion, and vagal mediation. Physiol. Behav. 56: 39-49.

Crawley, J. N. (1991) Cholecystokinin - dopamine interactions. Trends Pharmacol. Sci. 12: 232-236.

Crawley, J. N. and Corwin, R. L. (1994) Biological actions of cholecystokinin. Peptides 15: 731-755.

Della-Fera, M. A. and Baile, C. A. (1979) Cholecystokinin octapeptide: continuous picomole injection into the cerebral ventricles suppress feeding. Science 206: 471-473.

Denbow, D. M. and Myers, R. D. (1982) Eating, drinking and temperature responses to intracerebroventricular cholecystokinin in the chick. Peptides 3: 739-743.

Dentine, M. R. and Cowan, C. M. (1990) An analytical model for the estimation of chromosome substitution effects in the offspring of individuals heterozygous at a segregating marker locus. Theor. Appl. Genet. 79: 775-780.

Deschenes, R. J., Haun, R. S., Funkes, C. L. and Dixon, J. E. (1985) A gene encoding rat cholecystokinin. J. Biol. Chem. 260: 1280-1286.

Deschenes, R. J., Lorenz, L. J., Haun, R. S., Roos, B. A., Collier, K. I. and Dixon, J. E. (1984) Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proc. Natl. Acad. Sci. USA 81: 726-730.

Deschodt-Lanckman, M. (1982) Characterization of membrane-bond CCK-8 degrading enzymes from rat brain. Arch. Int. Physiol. Biochem. 90: B107-B108.

Dockray, G. J, Gregory, R. A. J. and Hutchison, J. B. (1978) Isolation, structure and biological activity to two cholecystokinin octapeptides from sheep brain. Nature 274: 711-713.

Dockray, G. J., Gregory, R. A., Tracy, H. J. and Zhu, W. Y. (1981) Transport of cholecystokinin-octapeptide-like immunoreactivity toward the gut in afferent vagal fibers in cat and dog. J. Physiol. Lond. 314: 501-511.

Ebenezer, I. S., De La Riva, C. and Baldwin, B. A. (1990) Effects of the CCK receptor antagonist MK-329 on food intake in pigs. Physiol. Behav. 47: 145-148.

Eberlein, G. A., Eysselein, V. E. and Goebell, H. (1988) Cholecystokinin-58 is the major molecular form in man, dog and cat but not in pig, beef and rat intestine. Peptides 9: 993-998.

Eberlein, G. A., Eysselein, V. E., Davis, M. T., Lee, T. D., Shively, J. E., Grandt, D., Niebel, W., William, R., Mosessner, J., Zeeh, J., Meyer, H. E., Goebell, H. and Reeve, J. R. (1992) Patterns of prohormone processing: order revealed by a new procholecystokinin - derived peptide. J. Biol. Chem. 267: 1517-1521.

Emson, P. C., Rehfeld, J. F. and Rossor, M. N. (1982) Distribution of cholecystokinin-like peptides in the human brain. J. Neurochem. 38: 1177-1179.

Eng, J. H., Du, B-H., Pan, Y., Chang, M., Hulmes, J. D. and Yalow, R. S. (1984) Purification and sequencing of a rat intestinal 22 amino-acid C-terminal CCK fragment. Peptides 5: 1203-1206.

Eysselein, V. E., Bottcher, W., Kauffman, G. L. and Walsh, J. H. (1984) Molecular heterogeneity of canine cholecystokinin in portal and peripheral plasma. Regul. Pept. 9: 173-185.

Eysselein, V. E., Eberlein, G. A. and Hesse, W. H. (1987) Cholecystokinin-S8 is the major circulating form of cholecystokinin in canine blood. J. Biol. Chem. 262: 214-217

Eysselein, V. E., Reeve, J. R., Shively, J. E., Miller, C. and Walsh, J. H. (1984) Isolation of a large cholecystokinin precursor from canine brain. Proc. Natl. Acad. Sci. USA 81: 6565-6568.

Falconer, D. S. (1989) Introduction to Quantitative Genetics. 3rd edition. Longman Scientific & Technical, Essex, England; John Wiley & Sons., New York.

Fan, Z. W., Eng, J. H., Miedel, M., Hulmes, J. D., Pan, Y.-C.E. and Yalow, R. S. (1987) Cholecystokinin octapeptides purified from chinchilla and chicken brains. Brain Res. Bull. 18: 757-760.

Figlewicz, D. P., Madzan, A. M., Sipols, A. J., Green, P. K., Liddle, R. A., Porte, D. and Woods, S. C. (1992) Intraventricular CCK-8 reduces single meal size in the baboon by interaction with type-A CCK receptors. Am. J. Physiol. 263: R863-R867.

Figlewicz, D. P., Schawartz, M. W., Seeley, R. J., Chavez, M., Baskin, D. G., Woods, S. C. and Porte, Jr. D. (1996) Endocrine regulation of food intake and body weight. J. Lab. Clin. Med. 127: 328-332.

Figlewicz, D. P., Sipols, A. J., Seeley, R. J., Chavez, M., Woods, S. C. and Porte, D. Jr. (1995) Intraventricular insulin enhances the meal-suppressive efficacy of intraventricular cholecystokinin octapeptide in the baboon. Behav. Neurosci. 109: 1-3.

Figlewicz, D. P., West, D., Stein, L. J., Woods, S. C. and Porte, D. Jr. (1986) Insulin alters the sensitivity of baboons to CCK-induced single meal suppression. Am. J. Physiol. 250: R856-R860.

Fine, N. C. and Winter, L. M. (1953) Selection for an increase in growth rate and market score in two inbred lines of swine. J. Anim. Sci. 12: 251.

Foster, J. A., Morrison, P. S., Dean, S. J., Hill, M. and Frank, K. (1980) Naloxone suppresses food/water consumption in the deprived cat. Pharmacol. Biochem. Behav. 14: 419-421.

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J. and MacLennan, D. H. (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448-451.

Funakoshi, A., Miyasaka, K., Jimi, T., Kawanami, T., Takata, Y. and Kono, A. (1994) Little or no expression of cholecystokinin A receptor gene in the pancrease of diabetic rats (Otsuka Long-Evans Tokushima Fatty--OLETF rats). Biochem. Biophys. Res. Commun. 199: 482-488.

Funakoshi, A., Miyasaka, K., Shinozaki, H., Masuda, M., Kawanami, T., Takata, Y. and Kono, A. (1995) An animal model of congenital defect of gene expression of cholecystokinin (CCK)-A receptor. Biochem. Biophys. Res. Commun. 21: 787-796.

Furness, J. B., Costa, M, Gibbins, I. L. and Llewellyn-Smith, I. J. (1985) Neurochemical similar myenteric and submucous neurons directly traced to the mucosa of the small intestine. Cell Tissue Res. 241: 155-163.

Gaisano, H. Y., Olivero, D., Reilly, W., Bouska, J., Roddy, D., Go, V. L. W. and Miller, L. J. (1984) Large molecular forms of cholecystokinin circulating in man. Gastroenterology 86: 1084.

Geola, F. L., Hershman, J. M., Warwick, R., Reeve, J. R., Walsh, J. H. and Tourtellotte, W. W. (1981) Regional distribution of cholecystokinin-like immunoreactivity in the human brain. J. Clin. Endocrinol. Metab. 53: 270-275.

Gibbs, J., Young, R. C. and Smith, G. P. (1973) Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psychol. 84: 488-495.

Go, V. L. W. and Owyang, C. (1980) Radioimmunoassays of cholecystokinin and gastric inhibitory peptide. In: Gastrointestinal Hormones. pp. 819-829. Edited by G. B. J. Glass. Raven Press, New York.

Goddard, C., Wilkie, R. S. and Dunn, I. C. (1988) The relationship between insulin-like growth factor-1, growth hormone, thyroid hormones and insulin in chickens selected for growth. Domest. Anim. Endocrinol. 5: 165-176.

Grawley, J. N. (1991) Cholecystokinin-dopamine interaction. Trends Pharmacol. Sci. 12: 232-236.

Greeley, G. H., Burdett, J. B., Hill, F. L. C., Spannagel, A., Trowbridge, J. and Thompson, J. C. (1984a) Distribution of cholecystokinin in the human alimentary canal. Gastroenterology 86: 1097.

Greeley, G. H. and Thompson, J. C. (1984b) Cholecystokinin-8 and neurotensin levels of plasma extracts and unextracted plasma. Fed. Proc. 43:1072-1076.

Green, E., Maffei, M., Braden, V., Proenca, R., DeSilva, U., Zhang, Y., Chua, S., Leibel, R., Weissenbach, J. and Friedman, J. (1995) The human obese (OB) gene: RNA expression pattern and mapping on physical, cytogenetic, and genetic map of chromosome 7. Genome Res. 5: 5-12.

Gregory, P. C., McFadyen, M. and Rayner, D. V (1989) Duodenal infusion of fat, cholecystokinin secretion and satiety in the pig. Physiol. Behav. 45: 1021-2024.

Grunfeld, C., Chao, C., Fuller, J., Pollock, A., Moser, A., Friedman, J. and Feingold, K. R. (1996) Endotoxin and cystokines induce expression of leptin, the *ob* gene product in hamsters. J. Clin. Invest. 97: 2152-2157.

Gubler, U., Chau, A. O., Hoffman, B. J., Collier, K. J. and Eng, J. (1984) Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. Proc. Natl. Acad. Sci. USA 81: 14307-14310.

Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K. and Friedman, J. M. (1995) Weight-reducing effects of the plasma protein encodeed by the obese gene. Science 269: 543-546.

Harper, A. A. and Raper, H. S. (1943) Pancreaozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J. Physiol. 102: 115-125.

Harro, J., Bvasar, E. and Bradwejun, J. (1993) CCK in animal and human research on anxiety. Trends Pharmacol. Sci. 14: 244-249.

Hastings, I. M., Bootland, L. H. and Hill, W. G. (1993) The role of growth hormone in lines of mice divergently selected on body weight. Genet. Res. 61: 101.

Hatfied, J. S., Millard, J. and Smith, C. J. V. (1974) Short-term influence of intraventromedial hypothalamic administration of insulin on feeding in normal and diabetic rats. Pharmacol. Biochem. Behav. 2: 223-226.

Hetherington, A. and Ranson, S. (1940) Hypothalamic lesions and adiposity in the cat. Anat. Rec. 78: 149-172.

Himick, B. A. and Peter, R. E. (1994) CCK/gastrin-like immunoreactivity in brain and gut, and CCK suppression of feeding in goldfish. Am. J. Physiol. 261: R841-R851.

Holtzman, S. G. (1974) Behavioral effects of separate and combined administration of naloxone and d-amphetamine. J. Pharmacol. Exp. Ther. 189: 51-55.

Horvat, H. and Medrano, J. F. (1995) Interval mapping of high growth (*hg*), a major locus that increases weight gain in mice. Genetics 139: 1737-1748.

Houpt, K. A., Houpt, T. R. and Pond, W. G. (1979) The pig as a model for the study of obesity and of control of food intake: a review. Yale J. Bio. Med. 52: 307-329.

Iida, M., Murakami, T., Ishida, K., Mizuno, A., Kuwajima, M. and Shima, K. (1996) Phenotype-linked amino acid alteration in leptin receptor cDNA from Zucker fatty (*fa/fa*) rat. Biochem. Biophys. Res. Commun. 222: 19-26.

Ingalls, A. M., Dickie, M. M. and Snell, G. D. (1950) Obesity, new mutation in the mouse. J. Hered. 41: 317-318.

Innis, R. B. and Snyder. S. H. (1980) Distinct cholecystokinin receptors in brain and pancreas. Proc. Natl. Acad. Sci. USA 77: 6917-6921.

Ivy, A. C. and Oldberg, E. (1928) A hormone mechanism of gallbladder contraction and evacuation. Am. J. Physiol. 86: 599-613.

Jansen, J. B. M. J. and Lamers, C. B. H. W. (1983) Radioimmunoassary of cholecystokinin in human tissue and plasma. Clin. Chim. Acta 131: 305-316.

Jensen, R. T., Lemp, G. F. and Gardner, J. D. (1982) Interactions of COOH-terminal fragments of cholecystokinin with receptors on dispersed acini from guinea pig pancreas. J. Biol. Chem. 257: 5554-5559.

Johnson, A. H. and Rehfeld, J. F. (1992) Identification of cholecystokinin/gastrin peptides in frog and turtle. Eur. J. Biochem. 207: 419-428.

Jorpes, E. and Mutt, V. E. (1966) Cholecystokinin and pancreozymin one single hormone ? Acta Physiol. Scand. 66: 196-202.

Joyner, K., Smith, G. P. and Gibbs, J. (1993) Abdominal vagotomy decreases the satiating potency of CCK-8 in sham and real feeding. Am. J. Physiol. 264: R912-R926.

Jung, Y. C., Rothschild, M. F., Flanagan, M. P., Christian, L. L. and Warne, C. M. (1989) Association of restriction fragment length polymorphisms of swine leucocytoantigen class I gene with production trait of Duroc and Hampshire boars. Anim. Genet. 20: 79-91.

Kaiyala, K. J., Woods, S. C. and Schwartz, M. W. (1995) New model for the regulation of energy balance and adiposity by the central nervous system. Am. J. Clin. Nutr. 62(suppl): 1123S-1134S.

Kamilaris, T. C., Johnson, E. O., Calogero, A. E., Kalogeras, K. T., Bernardin, R., Chrousos, G. P. and Gold, P. W. (1992) Cholecystokinin-octapeptide stimulates hypothalamic-pituitary-adrenal function in rats: role of corticotropin-releasing hormone. Endocrinology 130: 1764-1774.

Kanayama, S. and Liddle, R. A. (1991) Regulation of intestinal cholecystokinin and somatostatin mRNA by bombesin in rats. Am. J. Physiol. 261: G77-G81.

Karlsson, S. and Ahren, B. (1992) CCK-8 stimulated insulin secretion in vivo , is mediated by CCK-A receptors. Eur. J. Pharmacol. 213: 145-146.

Kawano, K., Hirashima, S., Mori, S., Saitoh, Y., Kurosumi, M. and Natori, T. (1992) Spontaneous long-term hypoglycemic rat with diabetic complications. Diabetes 41: 1422-1428.

Keightley, P. D., Hardge, T., May L. and Bulfield, G. (1996) A genetic map of quantitative trait loci for body weight in the mouse. Genetics 142: 227-235.

Kissileff, H. R., Pi-Sunyer, F. X., Thornton, J. and Smith, G. P. (1981) C-terminal octapeptide of cholecystokinin decreases food intake in man. Am. J. Clin. Nutr. 34: 154-160.

Klueppelberg, U. G., Molero, X., Barrett, R. W. and Miller, L. J. (1990) Biochemical characterization of the cholecystokinin receptor on CHP211 human neuroblastoma cells. Mol. Pharmacol. 38: 159-163.

Kothary, P. C., Vinik, A. I., Owyang, C. and Fiddian-Green, R. G. (1983) Immunochemical studies of molecular heterogeneity of cholecystokinin in duodenal perfusates and plasma in human. J. Biol. Chem. 258: 2856-2863

Koulisher, D., Moroder, L. and Deschodt-Lanckman, M. (1982) Degradation of cholecystokinin octapeptide related fragments analogs by human and rat plasma in vitro. Regul. Pept. 4: 127-139.

Kuhlers, D. L. and Jungst, S. B (1992) Correlated responses in reproductive and carcass traits to selection for increased 200-day weight in Duroc swine. J. Anim. Sci. 70: 2707-2713.

Kwon, H. Y., Bultman, S. J., Loffler, C., Chen, W. J., Furdon, P. J., Powell, J. G., Usala, A. L., Wilkison, W., Hansmann, I. and Woychik, R. P. (1994) Molecular structure and chromosomal mapping of the human homolog of the *agouti* gene. Proc. Natl. Acad. Sci. USA 91: 9760-9764.

Lander, E. S. and Botstein, D. (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199.

Lander, E. S. and Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics 11: 241-247.

Larsson, L. I. and Rehfeld, J. F. (1979) Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res. 165: 201-218.

Lee, G. H., Prvenca, R., Montez, J. M., Carroll, K.., Darvishzadeh., Lee, I. and Friedman, J. M. (1996) Abnormal splicing of the leptin receptor. Nature 379: 632-635.

Leibowitz, S. F. (1990) Hypothalamic neuropeptide Y, galanin and amines: concepts of coexistence in relation to feeding behavior. Ann. NY. Acad. Sci. 6121: 221-235.

Leory, P., Dessolin, S., Villageois, P., Moon, B. C., Friedman, J. M, Ailhaud, G. and Dani, C. (1996) Expression of *ob* gene in adipose cells. J. Biol. Chem. 271: 2365-2368.

Le Roy, Naveau, P. J., Elsen, J. M. and Sellier, P. (1990) Evidence for new major gene influencing meat quality in pigs. Genet. Res. Camp. 55: 33-40.

Levan, G., Szpirer, J., Szpirer, C., Klinga, K., Hanson, C. and Islam, M. Q. (1991) The gene map of the Norway rat (*Rattus norvegicus*) and comparative mapping with mouse and man. Genomics. 10: 699-718.

Levine, A. S. and Morley, J. E. (1984) Neuropeptide Y: a potent inducer of consummatory behavior in rats. Peptide 5: 1023-1029.

Lewis, L. D. and William, J. A. (1990) Regulation of cholecystokinin secretion by food, hormone, and neural pathways in the rat. Am. J. Physiol. 258: G512-G518.

Liddle, R. A., Gertz, B. J., Kanayama, S., Beccaria, L., Gettys, T. W., Taylor, I. L., Rushakoff, R. J., William, V. C. and Coke, C. D. (1990) Regulation of pancreatic endocrine function by cholecystokinin: Studies with MK-329, a nonpeptide cholecystokinin receptor antagonist. J. Clin. Endocrinol. Metab. 70: 1312-1318.

Liddle, R. A., Goldfine, I. D., Rosen, M. S., Taplitz, R. A., and Williams, J. A. (1985) Cholecystokinin bioactivity in human plasma. J. Clin. Invest. 75: 1144-1152.

Liddle, R. A., Goldfine, I. D. and Williams, J. A. (1984) Bioassay of cholecystokinin in rats: effects of food, trypsin inhibitor and alcohol. Gastroenterology 87: 542-549.

Lignon, M. F., Bernad, N. and Martinea, J. (1991) Pharmacological characterization of type B cholecystokinin binding sites on the human JURKAT T lymphocyte cell line. Mol. Pharmacol. 39: 615-620.

Lilja, P., Fried, G. M., Wiener, I., Inoue, K. and Thompson, J. C. (1982) Comparison of concentrations of plasma cholecystokinin in pig, dog and man after food or intraduodenal fat. Gastroenterology 82: 1118.

Logsdon, C. D. (1986) Glucocorticoids increase cholecystokinin receptors and amylase secretion in pancreatic acinar AR42J cells. J. Biol. Chem. 261: 2096-2101.

Lonovics, J., Guzman, S., Devitt, P. G., Hejtmancik, K. E., Suddith, R. L., Rayford, P. L. and Thompson, J. C. (1981) Action of pancreatic polypeptide on exocrine pancreas and on release of cholecystokinin and secretin. Endocrinology 108: 1925-1930.

Lotti, V. J. and Chang, R. S. L. (1989) A potent and selective nonpeptide gastrin antagonist and brain(CCKB) receptor ligand: L-365,260. Eur. J. Pharmacol. 163: 273-280.

Lu, D., Willard, D., Patel, I. R., Kadwell, S., Overton, L., Kost, T., Luther, M., Chen, W., Woychick, R. P., Wilkison, W. O. and Cone, R. D. (1994) Agouti protein is an antagonist of the melanocyte-stimulatin-hormone receptor. Nature 371: 799-802.

Lyon, M. F. and Searle, A. G. (1990) Catalog of mutant genes and polymorphic loci. In: Genetic variants and strains of the laboratory mouse. pp. 17-20. Oxford University Press, Oxford.

Maton, P. N., Selden, A. C. and Chadwick, V. S. (1982) Large and small forms of cholecystokinin in human plasma: measurement using high pressure liquid chromatography and radioimmunoassay. Regl. Pept. 4: 251-260.

McGowan, M. K., Andrews, K. M. and Grossman, S. P. (1992) Chronic intrahypothalamic infusion of insulin or insulin antibodies alter body weight and food intake in the rat. Physiol. Behav. 51: 753-766.

McKnight, B. J. and Goddard, C. (1989) The effect of food restriction on circulating insulin-like growth factor-I in mice divergently selected for high or low protein or fat to body mass ratios. Comp. Biochem. Physiol. 92A: 565-569.

McLaughlin, C. L. and Baile, C. A (1980) Decreased sensitivity of Zucker obese rats to the putative satiety agent cholecystokinin. Physiol. Behav. 25: 543-548.

McLaughlin, C. L. and Baile, C. A. (1981) Obese mice and the satiety effects of cholecystokinin, bombesin and pancreatic polypeptides. Physiol. Behav. 26: 433-437.

McLaughlin, C. L., Baile, C. A., Della-Fera, M. A. and Kasser, T. G. (1985) Meal-stimulated concentrations of CCK in the hypothalamus of Zucker obese and lean rats. Physiol. Behav. 35: 215-220.

Micelli, M. O. and Malsbury, C. W. (1983) Feeding and drinking response in golden hamster following treatment with cholecystokinin and agiotensin II. Peptides 4: 103-107.

Michaud, E. J., Bultman, S. J., Klebig, M. L., van Vugut, M. J., Stubbs, L. J., Russell, L. B. and Woychik, R. P. (1994) A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellows (A^Y) mutation. Proc. Natl. Acad. Sci. USA. 91: 2562-2566.

Milan, D., Le Roy, P., Woloszyn, N., Caritez, J. C., Elsen, J. M., Sellier, P. and Gellin, J. (1995) The RN locus for meat quality maps to pig chromosome 15. Genet. Sel. Evol. 27: 195-199.

Miller, L. J., Jardine, I, Weissman, E., Go, V. L. W. and Speicher, D. (1984) Characterization of cholecystokinin from the human brain. J. Neurochem. 43: 835-840.

Miller, M. W., Duhl, D. M. J., Vrieling, H., Cordes, S. P., Ollmann, M. M., Winkes, B. M. and Barsh, G. S. (1993) Cloning of the mouse *agouti* gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. Genes Dev. 7: 454-467.

Miyasaka, K., Kanai, S., Ohta, M., Kawanami, T., Kono, A. and Funakoshi, A. (1994) Lack of satiety effect of cholecystokinin (CCK) in a new rat model not expressing the CCK-A receptor gene. Neurosci. Lett. 180: 143-146.

Moos, A. B., McLaughlin, C. L. and Baile, C. A. (1982) Effects of CCK on gastrointestinal function in lean and obese Zucker rats. Peptides 3: 619-622.

Morley, J. E. (1985) Species differences in the response to cholecystokinin. Ann. NY Acad. Sci. 448: 413-416.

Morley, J. E. (1987) Neuropeptide regulation of appetite and weight. Endocr. Rev. 8: 256-286.

Morley, J. E. (1995) The role of peptides in appetite regulation across species. Amer. Zool. 35: 437-445.

Morley, J. E. and Levine, A. S. (1983) Involvement of dynorphin and Kappa opioid receptor in feeding. Peptides 4: 797-783.

Morley, J. E., Levine, A. S., Gosnell, B. A. and Billongton, C. J. (1984) Which opioid receptor mechanism modulates feeding. Appetite 5: 61-66.

Morley, J. S., Tracy, J. H. and Gregory, R. A. (1965) Structure-function relationships in the active C-terminal tetrapeptides sequence of gastrin. Nature 207: 1356-1359.

Murakami, T. and Shima, K. (1995) Cloning of rat obese cDNA and its expression in obese rats. Biochem. Biophys. Res. Commun. 209: 944-952.

Mutt, V. (1980) Cholecystokinin: isolation, structure and function. In: Gastrointestinal Hormones. pp. 169-203. Edited by G. B. J. Glass. Raven Press, New York.

Mutt, V. and Jorpe, J. E. (1968) Structure of porcine cholecystokini-pancreozymin: cleavage with thrombin and with trypsin. Euro. J. Biochem. 6: 156-162.

Mutt, V. and Jorpes, J. E. (1971) Hormonal polypeptides of the upper intestine. Biochem. J. 125: 57-58.

Mutt, V., Tatemota, K., Carlquist, M and Light, A. (1981) Cleavage of cholecystokinin with enterokinase. Biosci. Rep. 1: 651-659.

Nadeau, J. H. (1989) Maps of linkage and synteny homologies between mouse and man. Trends Genet. 5: 82-86.

Nash, W. G. and O'Brien, S. J. (1982) Conserved subregions of homologous G-bonded chromosomes between orders in mammalian evolution: carnivores and primates. Proc. Natl. Acad. Sci. USA 79: 6631-6635.

Neuenschwander, S., Rettenberger, G., Meijerink, E, Jorg, H. and Stranzinger, G. (1996) Partial characterization of porcine obesity gene (OBS) and its localization to chromosome 18 by somatic cell hybrids. Anim. Genet. 27 : 275-278.

Norton, S. A., Zavy, M. T., Maxwell, C. V., Buchanan, D. S. and Breazile, J. E. (1989) Insulin, growth hormone, glucose, and fatty acids in gilts selected for rapid vs slow growth rate. Am. J. Physiol. 257: E554-E560.

O'Brien, S. J. and Graves, J. A. M. (1991) Report of the committee on comparative gene mapping. Cytogenet. Cell Genet. 58: 1124-1151.

O'Brien, S. J., Seuanex, H. N. and Womack, J. E. (1988) Mammalian genome organization: an evolutionary overview. Ann. Rev. Genet. 22: 323-351.

Parrott, R. F. (1993) Peripheral and central effects of CCK receptor agonists on operant feeding in pigs. Physiol. Behav. 53: 367-372.

Pearson, R. K., Hadac, E. M. and Miller. L. J. (1989) Structural analysis of a distinct subtype of CCK receptor on human gastric smooth muscle tumor. Am. J. Physiol. 256: G1005-G1010.

Pekas, J. C. and Trout, W. E. (1993) Cholecystokinin octapeptide immunization: effect on growth of barrows and gilts. J. Anim. Sci. 71: 2499-2505.

Pelleymount, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winter, D., Boone, T. and Collins, F. (1995) Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269: 540-543.

Piper, L. R., Bindon, B. M. and Davis, G. H. (1985) The single gene inheritance of the high litter size of the Booroola Merino. In: Genetics of Reproduction in Sheep. pp. 115-125. Edited by R. B. Land and D. W. Robinson. Butterworth, London.

Plata-Salaman, C. R. and Oomura, Y. (1986) Effect of intra-third ventricular administration of insulin on food intake after food deprivation. Physiol. Behav. 37: 735-739.

Pomp, D., Cushman, M. A., Foster, S. C., Drudik, D. K. and Fortman, M. (1994) Identification of quantitative trait loci for body weight and body fat in mice. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production XXI: pp. 209-212. University of Guelph, Guelph, Canada.

Presti, M. E. and Gardner, J. D. (1993) Receptor antagonists for gastrointestinal peptides. Am. J. Physiol. 264: G399-G406.

Reeve, J. R., Eysselein, V. E., Walsh, J. H., Avram-Ben, C. M., and Shiveley, J. E. (1986) New molecular forms of cholecystokinin---microsequence analysis of forms previously characterized by chromatographic methods. J. Biol. Chem. 261: 16392-16397.

Rehfeld, J. F. (1978) Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J. Biol. Chem. 253: 4022-4030.

Rehfeld, J. F., Larsson, L. I., Goltermann, N. R., Schwartz, T. W., Holst, J. J., Jensen, S. L., and Morley, J. S. (1980) Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK. Nature 284: 33-38.

Rathje, T. A., Rohrer G. A. and Johnson, P. K. (1997) Evidence of quantitative trait loci affecting ovulation rate in pigs. J. Anim. Sci. (in press)

Rehfeld, J. F. and Lundberg, J. M. (1983) Cholecystokinin in feline vagal and sciatic nerves: concentration, molecular form and transport velocity. Brain Res. 275: 341-347.

Reidelberger, R. D. (1994) Cholecystokinin and control of food intake. J. Nutr. 124: 1327S-1333S.

Rentsch, J., Levens, N. and Chiesi, M. (1995) Recombinant *ob* gene product reduces food intake in fasted mice. Biochem. Biophys. Res. Commun. 214: 131-136.

Rettenberger, G., Bruch, J., Fries, R., Archibald, A. L. and Hameister, H. (1996) Assignment of 19 porcine type I loci by somatic cell hybrid analysis detects new regions of conserved synteny between human and pig. Mamm. Genome 7: 275-279.

Riedy, C. A., Chavez, M., Figlewicz, D. P. and Woods, S. C. (1995) Central insulin enhances sensitivity to cholecystokinin. Phyiol. Behav. 58: 755-760.

Ritter, R. C., Brenner, L. A. and Tamura, C. S. (1994) Endogenous CCK and the peripheral neural substrates of intestinal satiety. Ann. NY Acad. Sci. 713: 255-267.

Rohrer, G. A., Alexander, L. J., Keele, J. W., Smith, T. P. and Beattie, C. W.. (1995) A microsatellite linkage map of the porcine genome. Genetics 136: 231-245.

Rothschild, M. F., Hoganson, D. L., Warner, C. W. and Schwartz, N. K. (1990) The use of major histocompatibility complex class I restriction fragment length polymorphism analysis to predict performance in pig. In: Proceedings of the 4th World Congress on Genetics Applied to Livestock Production XII: pp. 125. Edinburgh, Scotland.

Rothschild, M. F., Liu, H. C., Tuggle, C. K., Yu, T. P. and Wang, L. (1995) Analysis of pig chromosome 7 genetic markers for growth and carcass performance traits. J. Anim. Breed Genet. 112: 341-348.

Rothschild, M. F., Jacobson, C., Vake, D., Tuggle, C., Wang, L., Short, T., Eckardt, G., Sasaki, S., Vincent, A., McLaren, D., Southwood, O., van der Steen, H., Mileham, A. and Plastow, G. (1996) The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc. Natl. Acad. Sci. USA 93: 201-205.

Sacerdote, P., Wiedermann, C. J., Wahl, L. M., Pert, C. B. and Ruff, M. R. (1991) Visualization of cholecystokinin receptors on a subset of humnan monocytes and in rat spleen. Peptides 12: 167-176.

Saito, A., Sankaran, H., Goldfine, I. D. and Williams, J. A. (1981) Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex. J. Neurochem. 37: 483-490.

Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Stael, B. and Auwery, J. (1995) Transient increase in obese gene expression after food intake or insulin administration. Nature 377: 527-529.

Sanacora, G., Kershaw, M., Finkelstein, J. A. and White, J. D. (1990) Increased hypothalamic content of preproneuropeptide Y mRNA in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 127: 730-737.

Sanger, D. J., McCarthy, P. S. and Metcalf, G. (1981) The effects of opiate antagonists on food intake are stereospecific. Neuropharmacology 20: 45-47.

Sankaran, H., Goldfine, I. D., Deveney, W. C., Wonga, K. Y. and William, J. A. (1980) Binding of cholecystokinin to high affinity receptors on rat pancreas. J. Biol. Chem. 255: 1849-1853.

Sasaki, S. Clutter, A. C. and Pomp, D. (1996) Assignment of the porcine obese (leptin) gene to chromosome 18 by linkage analysis of a new PCR-based polymorphism. Mammal. Genome 7: 471-472.

Sawyer, J. R. and Hozier, J. C. (1986) High resolution of mouse chromosomes: banding conservation between man and mouse. Science 232: 1632-1635.

Schultzberg, M., Hokfelt, T., Nilsson, G., Terentus, L., Rehfeld, J. F., Brown, M., Elde, R., Goldstein, M. and Said, S. (1980) Distribution of peptide- and catecholamine-containing neurons in the gastro-intestinal tract of rat and guinea-pig: immunohistochemical studies with antiserum to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine B-hydroxylase. Neuroscience 5: 689-744.

Shibasaki, T., Oda, T., Imaki, T., Ling, N. and Demura, H. (1993) Injection of anti-neuropeptide Y gamma-globulin into the hypothalamic paraventricular nucleus decrease food intake in rats. Brain Res. 601: 313-316.

Shimizu, H., Shargill, N. S., Bray, G. A., Yen, T. T. and Gesellchen, P. D. (1989) Effects of MSH on food intake, body weigh and coat color of the yellow obese mouse. Life Sci. 45: 543-552.

Silvente-Poirot, S., Dufresne, M., Vaysse, N. and Fourmy, D. (1993) The peripheral cholecystokinin receptors. Eur. J. Biochem. 215: 513-529.

Silver, A. J. and Morley, J. E. (1990) Role of CCK in regulation of food intake. Prog. Neurobiol. 36: 23-34.

Silver, A. J., Flood, J. F., Song, A. M. and Morley, J. E. (1989) Evidence for a physiological role for CCK in the regulation of food intake in mice. Am. J. Physiol. 256: R646-R652.

Sipols, A. J., Baskin, D. G. and Schwartz, M. W. (1995) Effects of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes 44: 147-151.

Siracusa, L. D. (1994) The *agouti* gene: turned on to yellow. Trends Genet. 10: 423-438.

Smith, G. P., Jerome, C., Cushin, B. J., Eterno, R. and Simansky, K. J. (1981) Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science 213: 1036-1037.

Smith, G. P., Jerome, C. and Norgren, R. (1985) Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am. J. Physiol. 249: R638-R641.

Soll, A. H., Amirian, D. A., Park, J., Elashoff, J. D. and Yamada, T. (1985) Cholecystokinin potentlly releases somatostatin from canine fundic mucisal cells in short-term culture. Am. J. Physiol. 248: G569-G573.

Soller, M. (1990) Genetic mapping of the bovine genome using DNA-level markers with particular attention to loci affecting quantitative traits of economic importance. J. Dairy. Sci. 73: 2628-2646.

Soller, M. (1994) Marker assisted selection - an overview. Anim. Biotech. 5: 193-207.

Soller, M. and Beckmann, J. S. (1982) Restriction fragment length polymorphisms and genetic improvement. In: Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production XI: pp. 396. Madrid, Spain.

South, E. H. and Ritter, R. C. (1988) Capsaicin application to central or perpheral vagal fiber attenuates CCK satiety. Peptide 9: 601-612.

Stanley, B. G., Kyrkouli, S. E., Lampert, S. and Leibowitz, S. F. (1986) Neuropeptide Y chronically injected into hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7: 1189-1192.

Stanley, B. G. and Leibowitz, S. F. (1984) Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 35: 2635-2642.

Stanley, B. G., Magdalin, W., Seifari, A. and Nguyen, M. M. (1992) Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. Peptides 13: 581-587.

Stephens, T. W., Basinski, M., Brisrow, P. K., Bue-Valleskey, J. M, Burgett, S. G., Graft, L., Hale, J., Hoffmann, J., Hsuing, H. M. and Kriauciunas, A. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377: 530-532.

Stone, R. T., Kappes, S. M. and Beattie, C. W. (1996) Bovine homolog of the obese gene maps to chromosome 4. Mamm. Genome 7: 399-400.

Straus, E., and Yalow, R. S. (1978) Species specificity of cholecystokinin in gut and brain of several mammalian species. Proc. Natl. Acad. Sci. USA 75: 486-489.
Straus, E. and Yalow, R. S. (1979) Cholecystokinin in the brains of obese and nonobese mice. Science 203: 68-69.

Strohmayer, R. A. L. and Smith, G. P. (1981) Cholecystokinin inhibits food intake in genetically obese (C57BL/6J-ob) mice. Peptides 2: 39-43.

Strubbe, J. H. and Mein, C. G. (1977) Increased feeding in response to bilateral injection of insulin antibodies in the VMH. Physiol. Behav. 19: 309-313.

Sun, G., Chang, T. M., Xue, W., Wey, J. F. Y., Lee, K. Y. and Chey, W. Y. (1992) Release of cholecystokinin and secretin by sodium in dogs: molecular form and bioactivity. Am. J. Physiol. 262: G35-G43.

Takahishi, Y., Kato, K., Hayashizaki, Y., Wakabayashi, T., Ohtsuka, E., Matsuki, S., Ikehara, M. and Matsubara, K. (1985) Molecular cloning of the human cholecystokinin gene by use of a synthetic probe containing deoxyinosine. Proc. Natl. Acad. USA 82: 1931-1935.

Takahashi, Y., Fukushige, S., Murosu, T. and Matsubara, K. (1986) Structure of human cholecystokinin gene and its chromosomal location. Gene 50: 353-360.

Tartaglia, L. A., Demski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Woolf, E. A., Monore, C. A. and Tepper, R. I. (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83: 1263-1271.

Tatemoto, K., Jornvall, H., Slimesmaa, S., Hallden, G. and Mutt, V. (1984) Isolation and characterization of cholecystokinin-58 (CCK-58) from porcine brain. FEBS Lett. 174: 289-293.

Trayhum, P. (1996) Expression of the *ob* (OBSES) gene and the leptin system. Int. J. Obesity. 20(supp.4): 22-23.

Tuggle, C. K., Schmitz, C. B., Wang, L. and Rothschild, M. F. (1995) Cloning and mapping of porcine OTF1 extends a synteny group conserved on SSC 4 and HSA 1. Mamm. Genome 6: 673-676.

Turkelson, C. M., Solomon, T. E. and Hamilton, J. (1990) A cholecystokinin metabolizing enzyme in rat intestine. Peptides 11: 213-219.

Vinayek, R., Jensen, R. J. and Gardner, J. D. (1987) Role of sulfate ester in influencing biologic activity of cholecystokinin-related peptides. Am. J. Physiol. 252: G178-G181.

Vitale, M., Vashishtha, A., Linzer, E., Powell, D. J. and Friedman, J. M. (1991) Molecular cloning of the mouse CCK gene: expression in different brain regions and during cortical development. Nucleic Acids Res. 19: 169-177.

Walson, J. H., Lamers, C. B. and Valenzuela, J. E. (1982) Cholecystokinin-octapeptide-like immunoreactivity in human plasma. Gastroenterology 82: 438-444.

Wangsness, P. J., Acker, W. A., Burdette, J. H., Krabill, L. F. and Vasilatos, R. (1981) Effect of fasting on hormones and metabolites in plasma of fast-growing lean and slow-growing obese pigs. J. Anim. Sci. 52: 69-74.

Wank, S. A. (1995) Cholecystokinin receptors. Am. J. Physiol. 269: G628-G646.

Wank, S. A., Pisgena, J. R. and de Weerth, A. (1992) Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc. Natl. Acad. Sci. USA 89: 8691-8695.

Webb, A. J., Carden, A. E., Smith, C. and Imlah, P. (1982) Porcine stree syndrome in pig breeding. In: Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production XI: pp. 41-51. Madrid, Spain.

Weller, J. I. (1996) Introduction of QTL detection and marker-assisted selection. In: XX Biotechnology's Role in the Genetic Improvement of Farm Animals. pp. 259-275. Edited by R. H. Miller, V. G. Pursel, and H. D. Norman. Beltsville Symposia in Agricultural Research. May 14-17, 1995. Published in 1996 by the American Society of Animal Science, Savoy, IL, USA.

Wilding, J. H. P., Gilbey, S. G. and Bailey, C. J. (1993) Increased neuropeptide Y messenger ribonucleic acid (mRNA) and decreased nruotensin mRNA in the hypothalamus of the obese(*ob/ob*) mouse. Endocrinology 132: 1939-1944.

Wilkie, P. J., Paszek, A. A., Flickinger, G. H., Rohrer, G. A., Alexander, L. J., Beattie, C. W. and Schook, L. B. (1996) Scan of 8 porcine chromosomes for growth, carcass and reproductive traits reveals two likely quantitative trait loci. XXVth Internatl. Conf. Anim. Genet.: 187.

Willard, D. H., Bodnar, W., Harris, C., Kiefer, L., Nichols, J. S., Blanchard, S., Hoffman, C., Moyer, M., Burkhart, W., Weiel, J., Luther, M. A., Wilkison, W. O. and Rocque, W. J. (1995) *Agouti* structure and function: characterization of a potent α-melanocyte stimulating hormone receptor antagonist. Biochemistry 34: 12341-12346.

Winkelman, D. C. and Hodgetts, R. B. (1992) RFLPs for somatotropic genes identify quantitative trait loci for growth in mice. Genetics 131: 929-937.

Woltmann, M. D., Clutter, A. C., Buchanan, D. S. and Dolezal, H. G. (1992) Growth and carcass charateristics of pigs selected for fast or slow gain in relation to feed intake and efficiency. J. Anim. Sci. 70: 1049-1059.

Woltmann, M. D., Clutter, A. C. and Buchanan, D. S. (1995) Effect of divergent selection for postweaning average daily gain on front-end soundness of market-weight pigs. J. Anim. Sci. 73: 1940-1947.

Womack, J. E. (1996) Integrating the syntenic, physical, and linkage maps. In: XX Biotechnology's Role in the Genetic Improvement of Farm Animals. pp. 91-102. Edited by R. H. Miller, V. G. Pursel, and H. D. Norman. Beltsville Symposia in Agricultural Research. May 14-17, 1995. Published in 1996 by the American Society of Animal Science, Savoy, IL, USA.

Woods, S. C. and Gibbs, J. (1989) The regulation of food intake by peptides. Ann. NY Acad. Sci. 575: 236-243.

Woods, S. C., Lotter, E. C., McKary, L. D. and Porte, D. Jr. (1979) Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboon. Nature 282: 503-505.

Woods, S. C. and Porte, D. Jr. (1976) Insulin and the set-point regulation of body weight. In: Hunger: Basic Mechanisms and Clinical Implication. pp. 273-280. Edited by D. Novin, D, G. A. Bray and W. Wyrwinchka. Raven Press, New York.

Yen, T. T., Gill, A. M., Frigeri, L. G., Barsh, G. S. and Wolff, G. L. (1994) Obesity, diabetes, and neoplasia in Yellow A^{vy} /-mice: ectopic expression of the *agouti* gene. FASEB J. 8: 479-488.

Yu, T. P., Tuggle, C. K., Schmitz, C. B. and Rothschild, M. F. (1995) Association of PIT polymorphisms with growth and carcass traits in pigs. Anim. Sci. 73: 1282-1288.

Zarbin, M. A., Wamsley, J. K., Innis, R. B. and Kukar, M. J. (1981) Cholecystokinin receptors: presence and axonal flow in the rat vagus nerve. Life Sci. 29: 697-705.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J. M. (1994) Positional cloning of the mouse *obese* gene and its human homologue. Nature 372: 425-432.

Zhou, Z. Z., Eng, J., Pan, Y., Chang, M., Hulmes, J. D., Raufman, J. P. and Yalow, R. S. (1985) Unique cholecystokinin peptides isolated from guinea pig intestine. Peptides 6: 337-341.

Zucker, K. A., Adrian, T. E., Bilchik, A. J. and Modlin, I. M. (1989) Effects of the CCK-receptor antagonist L364,718 on pancreatic growth in adult and developing animals. Am. J. Physiol. 257: G511-G516.

Zucker, L. M and Zucker, T. F. (1961) Fatty, a new mutation in the rat. J. Hered. 52: 275-278.

VITA

RUHONG JIANG

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE CONTRIBUTION OF CHOLECYSTOKININ TO APPETITE REGULATION IN PIGS WITH DIVEGENT GENETIC POTENTIAL FOR GROWTH AND FEED INTAKE

Major Field: Animal Breeding and Reproduction

Biographical:

Personal Data: Born in Taiyuan, Shanxi Province, China, June 22, 1964, the son of Danian Jiang and Shuzheng Qi.

Education: Graduated from NO. 1 Railway High School, Taiyuan, Shanxi Province, in July 1982; received Bachelor of Science in Biological Sciences from Fudan University, Shanghai, in July 1986; received Master of Science in Reproductive Physiology from Beijing Agricultural University, Beijing, in July 1991; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1997.

Experience: Graduate research and teaching assistant, Department of Animal Science, Oklahoma State University, 1993-1997; Instructor, School of Biological Sciences, Beijing Agricultural University, 1991-1993; Research Associate, School of Biological Sciences, Beijing Agricultural University, 1986-1991.

Professional Organizations: American Society of Animal Science, American Association for the Advancement of Science.