Understanding Obesity through a Comparison of Osborne-Mendel and S5B/P1 Strains of Rat

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ABSTRACT

Peripheral Ingestive Signaling Pathways

Peripheral Signaling

Nagase et al. (1996) compared the effects of exogenous pyruvate and lactate on the activity of hepatic pyruvate dehydrogenase (PDH), pyruvate, lactate, insulin, alanine, and glucose serum levels in O-M rats and S5B/P1 rats. Although the effects were short lived, O-M rats demonstrated higher serum pyruvate and serum lactate levels, but lower glucose levels. After exogenous pyruvate administration, pyruvate and lactate levels were greater in S5B/P1 rats than O-M rats. There was no difference in alanine levels and total PDH levels in respect to strain or diet differences. However, PDH in its active form was lessened in S5B/P1 rats. Insulin and hepatic PDH activity was increased in O-M rats fed both high- and low-fat diets. These effects were miniscule or absent in the S5B/P1 strain (Nagase, Bray, and York, 1996).

Schemmel et al. (1982) tested the S5B/P1 and O-M strains’ susceptibility to obesity when they were ovariectomized, injected with insulin, and put on a liquid sucrose diet. Ovariectomized rats of both strains gained 22% more weight than sham-operated controls. However, a loss in body weight and a decrease in food intake were observed after replacement estradiol was injected into the rats. The O-M rats that drank the sucrose solution gained more body fat and had higher levels of serum immunoreactive insulin, but had lower levels of serum free fatty acids. However, the sucrose solution suppressed weight gain in S5B/P1 rats (Schemmel, Teague, and Bray, 1982).

Lea-Currie et al. (1997) determined the effects of chronic dehydroepiandrosterone-sulfate (DHEAS) administration on adiposity in high- and low-
fat fed O-M rats. DHEAS had no effect on weight gain, water intake, or food consumption. However, high-fat fed O-M rats exhibited smaller fat pads with fewer adipocytes and carcass lipids. Low-fat fed O-M rats exhibited similar levels of adipose tissue mass and cellularity (Lea-Currie, Wen, and McIntosh, 1997).

Post-ingestive Feedback

Fisler et al. (1993) measured the effects of d-Fenfluramine in O-M and S5B/P1 rats fed a low-fat or high-fat diet. Overall, there was a slightly greater suppression of food intake in the O-M rats. Both O-M and S5B/P1 rats fed a low-fat diet demonstrated no difference in the effects of d-Fenfluramine. When O-M rats were fed a high-fat diet, the excess food intake and weight gain was completely eliminated. When fed a high-fat diet, there was an observed increase in purine nucleotide (GDP) binding in S5B/P1 rats in comparison with the O-M strain. When fed a low-fat diet, both strains exhibited an increase in nucleotide binding (Fisler, Underberger, York, and Bray, 1993).

Israel et al. (1993) examined the effect of diet-induced obesity on plasma insulin uptake into the CSF in female O-M rats. The dietary obese rats demonstrated an inverse effect of insulin uptake and plasma insulin levels. A decrease in CSF insulin uptake was observed as the plasma insulin levels increased (Israel, Park, Schwartz, Green, Sipols, Woods, Porte, and Figlewicz, 1993).

Fisler et al. (1983) measured the effect of drugs on food intake in both S5B/P1 and O-M strains of rats. Insulin and 2DG created a dose-dependent increase in food intake in both strains. However, S5B/P1 had a greater response with a shorter latency period than the O-M rats. High doses of d-amphetamine created a dose-dependent reduction in food intake in both strains. However at lower doses, d-amphetamine
increased food intake in O-M rats. Whereas the S5B/P1 rats were more affected by the anorectic effects of adenosine, the O-M rats were more sensitive to the higher dosages of adenosine. Naloxone affected both strains equally in reducing food intake, whereas d-glucose had no effect on food intake (Fisler and Bray, 1983).

Singer et al. (1997) investigated the responses of both S5B/P1 and O-M rats to mercaptoacetate (MA), a blocker of fatty acid oxidation, and 2-deoxy-D-glucose (2DG), a blocker of glucose utilization. MA increased food intake in O-M rats fed a high-fat diet and also stimulated carbohydrate and protein intake. On a low-fat diet, MA increased food intake in O-M rats. The S5B/P1 strain increased their food intake only at the maximum dosage of MA. 2DG increased food intake in both strains (Singer, York, and Bray, 1997).

*Fat Metabolism*

York et al. (1991) demonstrated the effects of injecting VPDPR, an amino terminal peptide of pancreatic procolipase, intraperitoneally into O-M rats. A reduction in food intake was observed only when the rats were fed a high-fat diet. Feeding inhibition was maintained as the VPDPR dose was increased. VPDPR inhibited fat intake, but had no effect on protein or carbohydrate intake (Okada, York, Bray, and Erlanson-Albertsson, 1991).

Okada et al. (1993) chronically infused enterostatin, a pentapeptide found in the procolipase molecule of the pancreas, into the lateral ventricle of O-M rats to determine its effects on food intake. Food intake, weight gain, and serum insulin levels were reduced upon enterostatin infusion. Corticosterone was increased after chronic infusion
and acute ICV injection. This elevation of corticosterone reduced the level of hepatic glucocorticoid receptor (Okada, York, and Bray, 1993).

Okada et al. (1992) measured the effect of enterostatin on food intake in fasted O-M and S5B/P1 rats. In a high-fat diet, there was an inhibition of overall food intake and fat intake in the O-M rats, but no effect was observed in the S5B/P1 rats. Pancreatic procolipase activity increased in S5B/P1 rats when fed both a low- and high-fat diet. There was a negative relationship between procolipase activity and food intake (Okada, York, Bray, Mei, and Erlanson-Albertsson, 1992).

**Taste**

Gilbertson et al. (2005) examined the responsiveness of fungiform taste receptor cells (TRCs) to free fatty acids (PUFAs) in both O-M and S5B/P1 strains of rats. S5B/P1 rats demonstrated an increased responsiveness to PUFAs, although both strains had similar inhibition constants. PUFAs enhanced the preference for saccharin in S5B/P1 rats, but not in O-M rats. O-M rats showed an increased density and expression of DRK currents. There was no difference in the fatty acid activated, two-pore domain, potassium channels among the strains (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005).

**Hypothalamic Ingestive Pathways**

**Neurotransmitters**

Kimbrough and Weekley (1984) investigated the effects of a low-fat and high-fat diet on brainstem and duodenal serotonin metabolism. Brainstem and duodenal levels of tryptophan, serotonin, and 5-HIAA were not affected by either diet. However, a high fat diet decreased brainstem levels of serotonin in the S5B/P1 rats. Brainstem and duodenal
levels of serotonin were decreased in O-M rats; this was not caused by dietary effects (Kimbrough and Weekley, 1984).

Weekley et al. (1982) demonstrated the effects of altered brain and systemic tryptophan metabolism in O-M and S5B/P1 rats. The O-M rats had a decrease in brainstem tryptophan 5-hydroxylase activity and 5-HT levels, but an increase in brain tryptophan levels. Pineal serotonin levels were also increased in the O-M strain as well as liver tryptophan 2, 3 dioxygenase activity. There was no difference in tyrosine aminotransferase activity, triglyceride levels, free fatty acid levels, and albumin levels between the strains. The O-M strain showed a decrease in total blood cholesterol, but an increase in unbound tryptophan levels. Total serum tryptophan was not different between the strains (Weekley, Maher, and Kimbrough, 1982).

Fisler et al. (1984) measured catecholamine turnover, potentially associated with norepinephrine or epinephrine turnover, in response to fasting, cold exposure, and diet in O-M and S5B/P1 rats. There was a greater concentration of interscapular brown adipose tissue and fractional norepinephrine turnover in fasted S5B/P1 rats than in fasted O-M rats. Cold exposure increased the fractional norepinephrine turnover rate in interscapular brown adipose tissue in both strains, but the O-M strain exhibited an increase in fractional norepinephrine turnover rate in the pancreas. Epinephrine turnover and adrenal concentration was similar in both strains. When fed a high-fat diet, there was a greater increase in the endogenous concentration of norepinephrine in interscapular brown adipose tissue in the S5B/P1 rats (Fisler, Yoshido, and Bray, 1984).

Fisler et al. (1989) measured the susceptibility of O-M and S5B/P1 rats to obesity through 3-OHB (3-OHB), glutamate, and GABA concentrations in the brain. 3-OHB and
glutamate concentrations were higher in S5B/P1 rats, unaffected by dietary fat; the S5B/P1 rats are exposed to higher levels of 3-OHB and glutamate than the O-M strain. GABA differed slightly between strains, but increased when dietary fat increased, especially in the S5B/P1 strain (Fisler, Shimizu, and Bray, 1989).

Shimizu et al. (1994) measured the norepinephrine, serotonin, and metabolite concentrations in the extracellular fluid of the ventromedial hypothalamus in O-M and S5B/P1 strains. They also measure monoamine alterations between the strains. Norepinephrine, serotonin, and 5-HIAA were lower in O-M rats when fed a low-fat diet. However, norepinephrine and serotonin levels increased when the O-M strain was given a high-fat diet; this eliminated the difference in amounts of neurotransmitters between the two strains. Ambient extracellular monoamines were lower in the O-M strain, but catecholamine response to dietary fat was greater in the O-M strain than in the S5B/P1 strain (Shimizu, Fisler, and Bray, 1994).

**Signaling**

Schaffhauser et al. (2002) conducted a study to determine the effects of dietary fat and genetics on the expression of hypothalamic genes involved in food intake. O-M rats displayed an increase in NPY expression and corticotrophin-releasing hormone in comparison with S5B/P1 rats. NPY-Y1 and NPY-Y5 receptor mRNA expression in the hypothalamus was significantly higher in O-M rats. When fed a high fat diet, the 5HT-2c receptor mRNA was significantly decreased in both O-M and S5B/P1 strains of rats (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002).

Bray et al. (1987) demonstrated the effects of high- and low-fat diets on 3-OHB and glucose transport across the blood brain barrier in both O-M and S5B/P1 rats. 3-
OHB uptake was higher in the S5B/P1 rats on both a high- and low-fat diet. Both strains demonstrated an increase in 3-OHB transport when fed a high-fat diet. There was no difference in glucose transport as a result of strain or diet effects (Bray, Teague, and Lee, 1987).

Arase et al. (1988) examined the effect of dietary fat on the response to 3-OHB and insulin infused chronically into the third ventricle in O-M and S5B/P1 rats. 3-OHB infusions decreased food intake and body weight in the O-M strains on both a high- and low-fat diet. The S5B/P1 were unaffected by the infusion. Insulin infusions decreased food intake and body weight in both of the strains fed a low-fat diet (Arase, Fisler, Shargill, York, and Bray, 1988).

Okada et al. (1992) investigated the effect of mifepristone (RU 486), a type II glucocorticoid receptor blocker, on obesity following a high-fat diet. High-fat fed O-M rats gained more weight and had larger retroperitoneal and parametrial fat pads in comparison to high-carbohydrate fed O-M rats. However, after RU 486 injections, these effects were eliminated. When fed a low fat diet and injected with RU 486, the O-M rats demonstrated a reduction in fat pad weight, but no effect on weight gain (Okada, York, and Bray, 1992).

Lin et al. (1996) determined the effects of NPY, β-casomorphin (an opioid-like peptide), and CRH on high- and low-fat food intake in O-M and S5B/P1 rats. Galanin was injected into the third cerebral ventricle and, in the O-M rats, stimulated a dose-dependent increase in food intake of both high-and low-fat diets, with the low-fat diet producing a smaller response than the high-fat diet. In the S5B/P1 strain, high-doses of galanin had a small stimulatory effect on food intake. β-casomorphin increased high-fat
food intake in the O-M strain, but had no effect on the S5B/P1 strain. There was no difference in NPY effects between the strains, with the exception that the S5B/P1 response was diminished. CRH’s anorectic effects were similar in both strains for both diets (Lin, York, and Bray, 1996).

**Lactation**

Using female O-M rats, Gerardo-Gettens et al. (1989) investigated the effects of prolactin on food intake and fat adipose tissue activity in the absence of progesterone. Food intake was higher in ovariectomized and hyperprolactinemic rats, whereas estrogen-treated rats demonstrated a decrease in food intake. There was no difference in adipose tissue activity among all rats. Prolactin stimulated food intake in the absence of ovarian progesterone (Gerardo-Gettens, Moore, Stern, and Horwitz, 1989).

**Exercise**

Hoffman-Goetz et al. (1983) measured the effect of treadmill exercise on food intake and body weight in S5B/P1 and O-M rats. Obese exercised males demonstrated a reduction in body weight with no change in food intake. Both lean and obese exercised females had no change in body weight or food intake (Hoffman-Goetz and MacDonald, 1983).

Applegate and Stern (1987) assessed the effects of chronic exercise 48, 60, 72, and 84 hours after exercise termination. Exercise depressed weight gain and light cycle food intake, whereas dark cycle food intake was unaffected. Exercise also depressed plasma insulin, epididymal and retroperitoneal depot weight and cell size, and retroperitoneal lipoprotein lipase activity (LPL). Forty-eight hours post-exercise termination, plasma insulin concentration increased to sedentary levels. Sixty hours post-exercise
termination, food intake increased above sedentary level and adipose LPL activity was similar to sedentary levels. Eighty-four hours post-exercise termination, dark-cycle food intake, plasma triglycerides, and epididymal LPL activity were increased above sedentary levels. This increase in food intake, plasma insulin, and enhanced LPL activity caused a preparatory response for rapid fat deposition after exercise termination (Applegate and Stern, 1987).

Applegate et al. (1982) compared O-M male and female weight gain, food intake, body composition, and blood lipids following a treadmill exercise program. The exercised male rats demonstrated a decrease in body weight and light cycle food intake, whereas females exhibited no change in food intake or body weight. In both males and females, body fat percentage was reduced, but only males had an increase in protein percentage. Both males and females showed a decrease in serum triglyceride levels, but only males showed a reduction in serum cholesterol (Applegate, Upton, and Stern, 1982).

Introduction

An individual’s body weight may be determined by genetics, metabolism, socioeconomic status, behavior, and the environment. Obesity is defined as a range of weight that is greater than what is considered healthy for a given height (Defining Overweight and Obesity, 2005). It is caused by an energy imbalance resulting from over-consumption of food and lack of exercise (Contributing Factors, 2005).

Obesity rates have risen across the world over the past twenty years. Initially, obesity trends were observed only in developed countries, but have now become a worldwide problem (Obesity Trends, 2005). Because obesity has become such an
overwhelming epidemic, national health programs have been implementde to reduce the frequency of obesity in the population from a prevalence of 40% to that of 15% (Obesity Trends, 2005). However, obesity rates have not reduced, but rather increased exponentially.

A major role in the increase of obesity is the environment and behavior. A typical diet consists of larger portions, higher fatty foods, and increased calorie consumption. The availability and convenience of such high-fat, high-sugar foods have made them vastly accessible to the population. The increase in obesity due to increased food intake could be prevented if people were equally as active (Contributing Factors, 2005).

Although technology has greatly benefited society, it has encouraged a more sedentary lifestyle through such innovations as computers, cars, and elevators. The body needs approximately 2,600 calories in order to sustain basal metabolism. Excess amounts of calories are stored by the body, causing weight gain. Physical activity is needed to compensate for the consumption of extra calories. Not only does a sedentary and food-indulgent lifestyle increase one’s likelihood of becoming obese, but genetics may also contribute to one’s susceptibility to obesity (Contributing Factors, 2005).

Genetic factors are an integral element in the development of and susceptibility to obesity. Much research has been done in order to understand the body’s mechanisms for fat recognition, fat intake, and developmental and hormonal plasticity in fat intake. Determining individual variances in obesity susceptibility will enable potential physiological and pharmacological treatments to be created for diet-induced obesity (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005). Because obese animal models have a preference for dietary fat, some areas of research have investigated the
relationship between the susceptibility to obesity and strain specific responses to ingestion in O-M and S5B/P1 strains of rats (Okada, York, Bray, and Erlanson-Albertsson, 1991). When fed a high-fat diet, O-M rats readily become obese. They prefer and consume more calories from a high-fat diet than from a high-carbohydrate or high-protein diet when given a three macronutrient taste-preference test (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002, and Nagase, Bray, and York, 1996). S 5B/P1 rats prefer a low-fat, and consume more calories from a high-carbohydrate diet (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002, and Nagase, Bray, and York, 1996). When fed a high-fat diet, initially the S5B/P1 readily eat, but quickly adapt and regulate their food intake (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002), experiencing only a slight increase in body weight (Bray, Teague, and Lee, 1987). Thus, they are referred to as being resistant to dietary-induced obesity (Fisler, Underberger, York, and Bray, 1993). Through comparing each strain’s physiological and behavioral characteristics and isolating the mechanisms involved in their susceptibility to diet-induced obesity, a quantifiable measurement of these mechanisms’ effects are provided (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002, and Arase, Fisler, Shargill, York, and Bray, 1988).

Both physiological and behavioral regulation is directed and manipulated by genetics, autonomic systems, and endocrine systems (Kimbrough and Weekley, 1984). There are strain specific responses to ingestion (Applegate and Stern, 1987), but these responses are only demonstrated when the rats have been subject to metabolic abnormalities initiated by a high-fat diet (Kimbrough and Weekley, 1984). Each strain’s susceptibility to diet-induced obesity is variable (Fisler, Yoshida, and Bray, 1984 and
Lin, York, and Bray, 1996) and their means for regulating energy balance is unknown (Arase, Fisler, Shargill, York, and Bray, 1988). Therefore, researchers are investigating the effects of peripheral ingestive signaling pathways, hypothalamic ingestive pathways, lactation, and exercise on the physiological and behavioral mechanisms involved in each strain’s susceptibility to obesity.

**Peripheral Ingestive Signaling Pathways**

The differences in the development of obesity between strains may be due to mechanisms involved in peripheral signaling, post-ingestive feedback, fat metabolism, and taste. Insulin and corticosterone concentrations are significantly higher in O-M rats, whereas blood serum and brain levels of β-hydroxybutyrate are lower in O-M rats. Low insulin levels promote the break-down of fatty acids and the production of ketones, and thus inhibit pyruvate and glucose oxidation. β-hydroxybutyrate reduces food intake and inhibits PDH activity via a vagally mediated pathway from the liver to the brain. The enzyme PDH is activated by insulin and converts pyruvate into acetyl coenzyme A through four different conversion pathways (Nagase, Bray, and York, 1996).

Ovariectomies and protamine zinc insulin injections stimulate weight gain and body fat accumulation due to the increase in hypoglycemia-induced food intake. When sucrose is substituted for glucose or starch, an increase in body fat has been observed (Schemmel, Teague, and Bray, 1982). Adipose tissue mass is influenced by adipocyte number and size. DHEAS effects are dependent upon dosage, route of administration, diet composition, and the rats’ genetic background. Because it reduces body fat and increases lean body mass, adipose tissue mass may be reduced by DHEAS, through
blocking adipogenesis, enhancing energy expenditure, or increasing lipid turnover. Chronic treatment of DHEAS decreases blood lipid levels, the deposition of adipose tissue, and fat synthesis (Lea-Currie, Wen, and McIntosh, 1997).

O-M rats also have 45% higher plasma insulin levels than their S5B/P1 counterparts, and are therefore classified as being hyperinsulinemic (Fisler and Bray, 1983). Plasma insulin uptake into CSF is decreased under fasting, free-feeding, hyperinsulinemia conditions. This reduced uptake of CSF insulin may be due to the decreased binding of insulin to isolated brain capillary preparations. Chronic elevation of plasma insulin may decrease binding of brain capillary insulin and also decrease the uptake of CSF insulin in obese-resistant rats. Circulating insulin from the pancreas may act as a feedback regulator to the CNS for food intake and body adiposity (Israel, Park, Schwartz, Green, Sipols, Woods, Porte, and Figlewicz, 1993).

After castration and VMH lesioning, S5B/P1 rats become obese. This suggests that the S5B/P1 strain of rats do not have a resistance to developing obesity, but rather they have a selective resistance to eating a high-fat diet. Therefore, Fisler et al. (1983) measured the behavioral response elicited by the following drugs: d-amphetamine, insulin, 2-deoxy-D-glucose (2DG), naloxone, glucose, and adenosine.

VPDPR is the amino terminal pentapeptide of pancreatic procolipase. It is activated in the small intestine; the activated peptide is called enterostatin, and is secreted after food ingestion. VPDPR produces an anorectic effect when injected into the peritoneum and third ventricle. Okada et al. (1992) measured the voluntary macronutrient choice of both O-M and S5B/P1 strains and their responses to enterostatin-induced effects on food. When orally administered, VPDPR inhibited feeding (Okada,
York, Bray, and Erlanson-Albertsson, 1991) and the taste preference for fat. Also, in attempts to produce antibodies to the enterostatin, weight loss occurs. Enterostatin also impairs the ability of perfused pancreatic islets to secrete insulin, suggesting that enterostatin has a direct effect on insulin secretion (Okada, York, Bray, and Erlanson-Albertsson, 1991). Therefore, enterostatin may inhibit the intake of fat and high-fat meals. Okada et al. (1993) suggested that chronic administrations of enterostatin would reduce food intake as well as body weight. Food intake can be stimulated by β-mercaptoacetate (MA) because of its tendency to inhibit free fatty acid oxidation. Therefore, Singer et al. proposed that S5B/P1 rats have impaired feeding responses to MA (Singer, York, and Bray, 1997).

Previous work has shown that S5B/P1 rats demonstrate a greater suppression of food intake when linoleic acid was infused into the duodenum. Taste stimuli transduction associates tastants and ion channels on the apical membranes of taste receptor cells (TRCs) which leads to depolarization of the cell and transmitter release. Delayed rectifying potassium (DRK) channels in the taste system are limited and specific to fatty acids and are involved in the repolarization of TRCs. Therefore, Gilbertson took a different approach and analyzed the effects of polyunsaturated free fatty acids (PUFAs) on DRK channels, reaching beyond the effects of textural properties and post-ingestive feedback signaling (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005).

**Peripheral Signaling**

Bray et al. (1996) hypothesized that PDH’s modulation of carbohydrate oxidation was related to a strain’s susceptibility to obesity when fed a high-fat diet. The proportion of and total activity of PDH, pyruvate, and lactate levels was measured in both fasted and
ad libitum-fed rats. An impairment in pyruvate clearance and metabolism in the liver of S5B/P1 rats may cause the lack of response to pyruvate or lactate. Both fasted O-M and S5B/P1 rats demonstrated no difference in serum lactate or pyruvate levels, but the ad libitum-fed S5B/P1 rats yielded higher serum pyruvate and lactate levels. Fasting eliminated these differences. Pancreatic colipase, the source of the peptide enterostatin which reduces food intake, is also lower in O-M rats. O-M rats had a greater reduction in food intake in response to intraperitoneal pyruvate and lactate administration on both a high- and low-fat diet in comparison to the S5B/P1 rats. The S5B/P1 rats demonstrated a reduced response to pyruvate when given a low-fat diet and no response when given a high-fat diet. Pyruvate’s ability to reduce food intake in the O-M rats but not in the S5B/P1 rats suggests the importance of the vagally mediated pathway in satiation. However, the S5B/P1’s lack of responsiveness to pyruvate and inability to suppress food intake after an increase in pyruvate levels suggests a resistance to generating the necessary vagal signals. Hepatic PDH activity was also similar among strains across diets, yet activated PDH was lower in the ad libitum-fed S5B/P1 rats, especially in those fed a high-fat diet (Nagase, Bray, and York, 1996).

Insulin concentrations in the O-M rats fed a high-fat diet were higher than both low-fat fed O-M and S5B/P1 and high-fat fed S5B/P1 rats, suggesting a difference in the clearing of pyruvate and lactate metabolites. The increase in insulin levels in the O-M rats is associated with an increase in pyruvate metabolism. Serum insulin levels were lower and β-hydroxybutyrate levels were higher in the ad libitum S5B/P1 rats. The low levels of insulin found in the S5B/P1 rats may enable them to maintain hepatic glycogen and blood glucose levels. This insulin and β-hydroxybutyrate combination inhibits PDH
activity and thus reduces pyruvate clearance levels. The lactate and pyruvate responses in the O-M strain were independent of their diet and therefore not caused by weight gain. These differences in insulin and $\beta$-OHB levels between strains may be the main contributing factor in their different metabolic responses to a high-fat diet. The higher ketone body levels of S5B/P1 rats slow the clearance of pyruvate (Nagase, Bray, and York, 1996).

Schemmel et al. (1982) determined the effects of ovariectomies, sucrose solution substitutions, and protamine zinc insulin injections on both adult virgin female O-M and S5B/P1 strains’ susceptibility to obesity. The ovariectomized rats demonstrated an increase in food intake and body weight. Estradiol injections however, reduced food intake and body weight. Therefore, when given replacement estrogen, ovariectomized rats regulated body weight at levels consistent with the control rats. It also increased the number of pancreatic and $\beta$-cells which reflects high levels of serum insulin. It is proposed that estradiol reduces the amount of fat in the total body mass by mobilizing it (Schemmel, Teague, and Bray, 1982).

The O-M rats gained more weight and had more fat accumulation in comparison to the S5B/P1 rats when given the sucrose solution to drink. The sucrose raised insulin levels which lowered free fatty acid levels and increased the development of adipose tissue. The O-M rats were able to adjust to the high levels of exogenous insulin, whereas the S5B/P1 rats were not. The S5B/P1 did not show an increase in body weight, had smaller numbers of fat cells, and experienced ineffective free fatty acid clearance from serum by insulin.
Lea-Currie et al. (1997) proposed that O-M rats were more responsive to the anti-obesity effects of DHEAS because DHEAS may block the activation of PPARγ2 and its ability to induce preadipocyte maturation. Because O-M rats gained more weight, had more carcass lipids, greater retroperitoneal, inguinal, and gonadal fat mass, and had higher numbers of adipocytes, it was concluded that they are more susceptible to diet-induced obesity. This increased susceptibility to dietary fat may be due to an over-expression of peroxisome proliferator activated receptors (PPARγ2) which may cause an increase in adipocyte number. Increased levels of PPARγ2 would promote the conversion of preadipocyte cells into mature lipid-filled cells. In O-M rats, DHEAS was ineffective in decreasing adiposity in low-fat, low-sugar, and high-starch diets. However, when fed a high-fat diet and given DHEAS, the O-M rats experienced an attenuation of adipose tissue. High-sugar diets also increased lipogenesis in the liver and adipocytes. DHEAS’s effectiveness in preventing or reducing adiposity may be dependent upon the sugar and fat content in the rat’s diet. Since DHEAS reduces lipogenesis as well as lipid synthesis, DHEAS administration may be most effective when a sucrose-rich diet is fed to the animal. The O-M rats given DHEAS had fewer carcass lipids, less fat depositions, reduced retroperitoneal and epididymal adipocytes. Although DHEAS increased energy expenditure, food and water intake and weight gain were similar in DHEAS-treated and control rats (Lea-Currie, Wen, and McIntosh, 1997).

O-M rats have lower sympathetic nervous system activity, lower levels of 3-OHB, and lower norepinephrine and serotonin levels in comparison to their S5B/P1 counterparts. The lower levels of serotonin imply an increased resistance to dietary fat-induced obesity in response to enhanced serotonin release. D-fenfluramine is specific for
the serotonergic system by inhibiting serotonin uptake and reducing the release of serotonin stores. Therefore, it was proposed that increasing serotonin levels in the hypothalamus would prevent dietary fat-induced obesity in the O-M strain of rats (Fisler, Underberger, York, and Bray, 1993).

Post-ingestive feedback

Serotonin levels in the brainstem and basal release of serotonin in O-M rats were lower than the levels found in the S5B/P1 rats. When d-fenfluramine was administered, its effects on macronutrient selection caused a greater reduction in food intake in rats eating a low-fat/high-carbohydrate diet. D-fenfluramine releases serotonin and prevents serotonin reuptake. It reduced food intake and increased sympathetic nervous system activity and thermogenesis in both strains of rats; these effects were independent of diet type. Fenfluramine, in combination with a high-fat diet, increased GDP binding and the firing rate of sympathetic neurons to brown adipose tissue in S5B/P1 rats. A single injection of d-fenfluramine increased serotonin in the VMH and LH while also increasing norepinephrine in the VMH. In O-M rats fed a high-fat diet, chronic treatment of d-fenfluramine eliminated excess weight gain and food intake. In the S5B/P1 strain, the reduction in food intake was brief and quickly returned to normal levels. Because O-M rats experienced an elimination of weight gain, it is suggested that d-fenfluramine inhibits the reward-system by increasing serotonin release in the LH of O-M rats but not in S5B/P1 rats. This reward system may project through the VTA, NTS (taste center), and LH. Electrical stimulation of the LH is excited by taste, metabolic factors, and insulin, and therefore, chronic feeding of a high-fat diet could result in a loss of sympathetic
nervous system activity, causing a decrease in food intake (Fisler, Underberger, York, and Bray, 1993).

A cafeteria diet, a diet of high caloric density, caused a 20% increase in body weight but did not alter plasma or CSF insulin uptake mechanisms. Female O-M rats had a greater uptake of insulin into the CSF relative to their male counterparts at comparable plasma levels. Chronically elevating ambient insulin levels resulted in hyperinsulinemia and a general decrease in CSF/plasma IRI uptake was observed. CSF insulin uptake decreased as the plasma immunoreactive insulin (IRI) levels increased. As plasma IRI uptake increases, the receptor mediated transcytosis component becomes saturated and the less efficient mechanism of diffusion becomes dominant. The receptor component may become saturated more readily in hyperinsulinemic rats. This would reduce the efficiency of insulin uptake into the CSF without changing the number of receptors.

Higher plasma IRI levels influence the binding of brain capillary insulin and CSF insulin uptake. The acceleration of insulin clearance may contribute to the decrease in plasma IRI uptake into the CSF and the increase in plasma IRI levels. The saturation of the insulin transport mechanism across the blood-brain barrier contributes to the uptake of circulating insulin into the brain. Regulating insulin access to the brain may be influenced by nutritional status, more so by a negative caloric balance than a positive caloric balance (Israel, Park, Schwartz, Green, Sipols, Woods, Porte, and Figlewicz, 1993).

Because O-M and S5B/P1 rats differ in the amount of weight gained from eating a high-fat diet, Fisler proposed that their responses would also differ in the degree to which particular drugs modified food intake and in their responsiveness of the hypothalamic
GABA-mediated transmitter system. Insulin and 2DG increased food intake, dependent upon the administered dose, in both O-M and S5B/P1 rats. However, the S5B/P1 rats demonstrated a greater response with a shorter latency duration to both insulin and 2DG. The higher latency period in relation to the onset of eating after insulin injections seen in the O-M rats may result from delayed glucopenia. This insulin-induced hyperphagia may be due to a peripheral signal of glucose utilization, glucoprivation of glucose sensitive cells in the hypothalamus caused by an increase in glucose utilization, or insulin may be directly acting on the hypothalamic insulin receptors. Chronically down-regulating the insulin receptors in the O-M rats resulted in slowed glucose uptake and utilization. Therefore, 2DG-induced hyperphagia may be caused by peripheral or central glucoprivation or 2DG’s interaction with norepinephrine. 2DG induces eating by directly acting on the brain directly through cellular glucopenia. When glucose levels were high in the blood, 2DG uptake was inhibited. In O-M rats, 2DG may stimulate a greater release of pancreatic glucagon and a greater production of hepatic glucose. The shorter latency observed in the S5B/P1 rats given 2DG suggests that there may be a difference in glucoprivation or hypoglycemia development between the strains. The heightened sensitivity to 2DG observed in S5B/P1 suggests that the differences in the strains’ responses to glucopenia are due to different target tissues in the brain (Fisler and Bray, 1983).

Fisler also determined that there is a strain difference in the α-adrenergic feeding system. The two adrenergic systems, alpha and beta, function in opposition to each other in regulating food intake. Since the action of amphetamines are caused by the release of the neurotransmitters dopamine and norepinephrine, feeding responses were stimulated
by injecting norepinephrine into the hypothalamus. D-amphetamine acts as antagonist for both of these systems by binding to the hypothalamic receptors. D-amphetamine stimulated food intake through the $\alpha$–adrenergic system, but suppressed food intake through the $\beta$–adrenergic system. The $\beta$–receptor system was more potent than the $\alpha$–receptor. Therefore high doses of d-amphetamine would inundate the $\alpha$–receptor response and cause a general suppression of food intake. Higher doses of d-amphetamine suppressed food intake at similar levels across both strains. Lower doses of d-amphetamine produced an increase in food intake in the O-M rats; this response was not displayed in the S5B/P1 rats (Fisler and Bray, 1983).

Because adenosine is produced and released from adipose tissue, subcutaneous injections of adenosine reduced food intake. Adenosine acts as a physiological signal to the hypothalamus of energy stores for regulating food intake. S5B/P1 rats were more sensitive to the anorectic effect of adenosine when administered in lower dosages. At higher doses, the O-M rats were more sensitive to adenosine’s anorectic effects. Naloxone, an opioid agonist, also suppressed food intake. Although naloxone suppressed food intake in both strains, S5B/P1 displayed a greater sensitivity and shorter latency to naloxone’s suppressive effects. It was surprising that the O-M strain were less responsive to naloxone because of the drug’s hypothalamic and genetic effects (Fisler and Bray, 1983).

In O-M rats, MA administration increased food intake independently of diet type; carbohydrate and protein intake was increased, but fat intake remained consistent. Both O-M and S5B/P1 strains were more sensitive to the effects of MA when they were maintained on a low-fat diet. The S5B/P1 rats increased food intake when fed a low-fat
diet in conjunction with MA administration. Because feeding is dependent upon the vagus nerve, S5B/P1 rats may have an impaired peripheral and central signaling system involved in reducing free fatty acid oxidation. A reduction in vagal nerve signaling may alter the response to fatty acid availability or reduce feeding intake (Singer, York, and Bray, 1997). Galanin, localized to the PVN, stimulates fat feeding (Okada, York, Bray, and Erlanson-Albertsson, 1991) and is involved in MA-induced feeding by antagonizing and thus reducing the feeding response to MA. Therefore, S5B/P1 rats may have an impaired galanin system that decreases their response to MA. However, MA may not reduce free fatty acid oxidation to the same extent in S5B/P1 rats as it does in the O-M strain. The S5B/P1 rats yielded a heightened sensitivity to circulating plasma free fatty acids which would reduce MA-induced feeding. MA may affect metabolic processes differently between strains such that plasma glucose levels are altered. S5B/P1 rats also exhibited higher brain and plasma levels of 3-OHB. 3-OHB is a metabolite of fatty acids that is increased in the blood and in the brain upon eating a high-fat diet. O-M rats displayed a decrease in food intake after administration of 3-OHB, whereas S5B/P1 rats demonstrated no response. This supports the proposal that S5B/P1 have an impaired response to free fatty acid signals (Singer, York, and Bray, 1997).

Fat Metabolism

When fat is ingested, VPDPR is released and circulated throughout the body. Okada suggests that the level of the circulating peptide reflects the level of dietary fat in a meal. In that case, VPDPR may act as a feedback system for regulating dietary fat intake. When injected peripherally, VPDPR suppressed feeding. Enterostatin, VPDPR in its activated form, may either produce a lengthy bodily response or may have a long
circulation half-life. Impaired secretion or response to enterostatin in the O-M rats may inhibit fat regulation and promote the development of obesity. Reduced VPDPR secretion after feeding may potentially contribute to hyperphagia and the development of a taste preference for fat. In addition, an adrenalectomy prevents the development of obesity by normalizing food intake while also enhancing the production and release of VPDPR (Okada, York, Bray, and Erlanson-Albertsson, 1991).

Chronic administrations of enterostatin into the third ventricle reduced food intake and weight gain in O-M rats fed a high-fat diet. Increased circulation of enterostatin suppressed weight gain and food intake in O-M rats fed a high-fat diet. Enterostatin also elicited changes in the endocrine system. Corticosterone concentrations were elevated thirty-times that of normal levels, suggesting that enterostatin activates the HPA-axis to stimulate CRH secretion. CRH also inhibits food intake, especially fat and protein intake. The glucocorticoid receptor count was reduced however in hepatic cystols; this down-regulation of glucocorticoid receptors was produced by circulating corticosterone. Enterostatin may also affect the glucose and insulin systems of the hypothalamus as well as autonomic nervous system output, causing a reduction in insulin levels. However, decreased insulin levels may be an effect of decreased food intake and weight gain (Okada, York, and Bray, 1993).

The S5B/P1 rats diet preference was unaffected by enterostatin, but had high levels of pancreatic colipase activity. O-M rats responded to the exogenous enterostatin by reducing their fat intake and experienced lower levels of pancreatic colipase activity. These low pancreatic colipase levels may be related to the O-M rats’ reduced secretion of the peptide. Maximal endogenous enterostatin activity may be the reason that no
response was elicited in the S5B/P1 strain of rats. Increased enterostatin secretion or increased target cell sensitivity to enterostatin may cause maximum colipase activity in the strain. There are three observations that support the maximum colipase activity explanation. Pancreatic colipase activity was higher yet they displayed a reduced intake of dietary fat. Pancreatic colipase activity was related to the response to exogenous enterostatin levels. Also, the S5B/P1 rats demonstrated high levels of pancreatic colipase, suggesting a high rate of endogenous enterostatin production. Therefore, high levels of endogenous enterostatin produce a maximum inhibition of fat intake (Okada, York, Bray, Mei, and Erlanson-Albertsson, 1992).

Taste

There are differences between S5B/P1 and O-M rats in the effects of free fatty acids on DRK channels, which may contribute to the strains’ expression of DRK channels. The greater FA-sensitive to FA-insensitive ratio found in S5B/P1 rats may account for the differences in the two strains’ responsiveness to fatty acids. These differences may contribute to the phenotypic differences in DRK current. O-M rats yielded a higher concentration of DRK channels and a higher DRK current, whereas S5B/P1 rats experienced a greater inhibition of DRK channels. DRK channels are principally fatty-acid sensitive in the S5B/P1 rats, whereas the majority of the DRK current in the O-M strain was not inhibited by PUFAs. It could be suggested that pre- and post-ingestive chemoreception input into a negative feedback pathway may control appetite. The O-M’s observed weaker chemosensory input indicate a diminished capacity in pathway activation and therefore an increase in their intake of dietary fat (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005).
Summary

Both strains were affected by the manipulations involved in the peripheral signaling of food intake. When fed a high-fat diet, rats’ susceptibility to obesity may be influenced by PDH modulation of carbohydrate oxidation (Nagase, Bray, and York, 1996). Rats given a sucrose solution had an increase in body fat and serum insulin levels, but lower serum free fatty acid levels. The sucrose solution suppressed weight gain in the S5B/P1 rats (Schemmel, Teague, and Bray, 1982). Low doses of DHEAS in combination with a high-fat diet reduced carcass lipids, fat deposition, and retroperitoneal and epididymal adipocytes. The anti-obesity effects of DHEAS were dependent upon the level of fat in the rats’ diet (Lea-Currie, Wen, and McIntosh, 1997). Because the high-fat diet, increases in GDP binding in the S5B/P1 rats, and blocked GDP binding caused by fenfluramine, fenfluramine is suggested to block the feeding reward system stimulated by a high-fat diet (Fisler, Underberger, York, and Bray, 1993). Female O-M rats had a greater uptake of insulin into the CSF relative to their male counterparts at comparable plasma levels. Insulin transport into CSF was not altered by moderate diet-induced obesity or hyperinsulinemia. An insulin transport system that initially uses receptor mediated channels and then, upon saturation, utilizes diffusion, provides the advantage of maintaining maximal sensitivity of insulin within the CNS to dramatic changes of ambient insulin levels (Israel, Park, Schwartz, Green, Sipols, Woods, Porte, and Figlewicz, 1993). O-M and S5B/P1 had different responses to the following three stimulatory drugs: 2DG, d-amphetamine, and naloxone; the responses demonstrated were dose-dependent (Fisler and Bray, 1983). Enterostatin inhibited fat intake in O-M rats on a high-fat diet, but had no effect on the low-fat diet. The S5B/P1 rats demonstrated no
change in response due to enterostatin administration, but did experience an increase in pancreatic colipase activity (Okada, York, Bray, Mei, and Erlanson-Albertsson, 1992). VPDPR inhibited fat intake without having an effect on carbohydrate or protein intake, and therefore, VPDPR may act as a feedback signal for regulating dietary fat intake (Okada, York, Bray, and Erlanson-Albertsson, 1991). Enterostatin, VPDPR in its activated form, reduced weight gain in the O-M rats when it was administered centrally into the brain. In addition to effects on feeding, enterostatin also affected insulin and corticosterone secretion in a way that would promote weight loss (Okada, York, and Bray, 1993). O-M and S5B/P1 rats had a similar response to 2DG, but had different responses to MA. Therefore, a difference in central and peripheral signaling systems involved in free fatty acid oxidation or a difference in metabolic environments may provide the difference in the strains’ susceptibility to obesity (Singer, York, and Bray, 1997). TRCs from S5B/P1 rats were more responsive to PUFAs than the O-M rats. However, the O-M rats had a greater density current and number of DRK channels. Therefore, the ratio of PUFA-sensitive channels to PUFA-insensitive channels may be the contributing factor to PUFA sensitivity and each strain’s response to dietary fat. The degree of DRK channel expression of each strain may also have contributed to the phenotypic differences observed in O-M and S5B/P1 rats and may also have been an important factor in shaping taste preferences and fat intake (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005).

_Hypothalamic Ingestive Pathways_
Feeding and metabolic processes are regulated by endocrine, autonomic, and genetic factors (Weekley, Maher, and Kimbrough, 1982). The hypothalamus plays an integral role in fat regulation and food intake through neurotransmitter and hormone production and release (Kimbrough and Weekley, 1984). Lesioning the VMH has induced hyperphagia and obesity, partially due to altered autonomic signaling in nerves innervating pancreatic islets (Weekley, Maher, and Kimbrough, 1982). The differences in the development of obesity between strains may be due to mechanisms involved in the action of hypothalamic neurotransmitters and hypothalamic signaling. O-M rats’ weight gain as been attributed to hypertrophy and hyperplasia. Serotonin acts as a satiety factor through the hypothalamus (Weekley, Maher, and Kimbrough, 1982) and because it is involved in feeding and weight regulation, the increase in body fat, in conjunction with an energy imbalance, may affect ingestive behaviors through its influence on tissue serotonin levels (Kimbrough and Weekley, 1984). Obesity alters the metabolism of plasma tryptophan and brain serotonin levels (Kimbrough and Weekley, 1984). Altered catecholamine levels have been observed in O-M rats; depleted levels may result in hypophagia.

Cold exposure, fasting, and overfeeding alter sympathetic nervous system activity with cold exposure and overeating acting as a stimulant and fasting as a suppressant. Therefore, a differing functional state in sympathetic nervous system activity between strains may influence food intake (Fisler, Yoshido, and Bray, 1984). By directly infusing norepinephrine or stimulating the LH, an increase in feeding occurs. Infusing serotonin (5-HT) into the LH may reduce feeding or produce no effect at all (Weekley, Maher, and Kimbrough, 1982). S5B/P1 rats have a higher concentration of norepinephrine turnover
in brown adipose tissue; an increase in norepinephrine turnover may be due to an increase in fat ingestion. 3-OHB acts through the brain to reduce food intake and body weight in O-M rats, independently of dietary fat intake. The effects of GABA on food intake are dependent upon the site of injection. Fisler et al. (1989) tested the levels of brain 3-OHB, glutamate, and GABA between O-M and S5B/P1 rats. In attempts to identify the differences in O-M and S5B/P1 responses to ambient hypothalamic serotonin, norepinephrine, and metabolites during high- and low-fat diets, Shimizu measured the amount of extracellular hypothalamic monoamines (Shimizu, Fisler, and Bray, 1994).

Since O-M and S5B/P1 rats respond differently to a high-fat diet, there is an increased possibility that respond differently to metabolic peptides that affect nutrient intake (Lin, York, and Bray, 1996). Such metabolic peptides include NPY, galanin, and growth-hormone releasing hormone which increase food intake, and corticotrophin-releasing factor, neurotensin, and cholecystokinin (CCK) which reduce food intake. NPY, mediated by the Y-5 and Y-1 receptors, is essential in controlling appetite and balancing energy intake. It stimulates carbohydrate intake when injected into the PVN. In addition to its stimulation of carbohydrate intake, NPY’s prevalence in both the S5B/P1 and O-M strain may explain some of the difference in the strains’ dietary preferences (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002). Galanin, localized to the PVN, stimulates fat feeding (Okada, York, Bray, and Erlanson-Albertsson, 1991). The active process of transporting these and other metabolites, such as ketones, glycerol, and fatty acids, across the blood-brain barrier and into the brain may be related to the strains’ susceptibility to obesity and their ability to reduce food intake (Bray, Teague, and Lee, 1987).
Glucocorticoids are also essential in the development of obesity. Their removal prevents hyperphagia, reduces insulin levels, fat synthesis and deposition, and restores sympathetic activity to brown adipose tissue. Mifepristone (RU 486) has been shown to eliminate obesity by blocking the receptors (Okada, York, and Bray, 1992).

**Neurotransmitters**

Serotonergic neurons in the gut have serotonergic receptors that produce serotonin from tryptophan by the enzyme tryptophan hydroxylase. This enzyme inhibits the cholinergic excitatory pathway. When the vagal nerve is stimulated, it releases serotonin. Because the hypothalamus regulates autonomic signals, this autonomic input into the gut may regulate serotonin levels, thus affecting duodenal motility and nutrient absorption. Therefore, a disturbance in brain and duodenum serotonin biosynthesis may affect the intestinal transit time of a meal in the O-M rats, thus contributing to their increased susceptibility to obesity.

Ingestion of a high-fat diet did not alter serotonin, 5-HIAA, and tryptophan levels in the brainstem or in the duodenum, and ingestion of a low-fat diet had no effect on serotonin levels. However, independent of diet, the O-M strain of rats demonstrated decreased serotonin and 5-HIAA levels, indicating an impaired tryptophan hydroxylating system. Serotonergic neurons may inhibit food intake in the O-M rats. Therefore, a reduction in brain 5-HT metabolism may cause hyperphagia and obesity (Kimbrough and Weekley, 1984).

The diabetic tendencies and susceptibility to obesity seen in O-M rats may be caused by recessive characteristics expressed through metabolic anomalies associated with ingesting a high-fat diet. CNS serotonergic neurons may inhibit food intake in O-M
rats, although dietary fat does not have an effect on serotonin levels. Diets high in polyunsaturated fats do not alter duodenal or brain 5-HT levels. However, when O-M rats are given a normal diet, their serotonin levels as well as serotonin synthesis decrease while tryptophan levels increase. This implies that, in conjunction with their preference for a high-fat diet, brainstem 5-HT synthesis is depressed in O-M rats which may induce hyperphagia and obesity. Therefore, the O-M rats may have a greater 5-HT turnover rate and increased serotonin catabolism. Although free serum tryptophan was increased in O-M rats, tryptophan hydroxylase activity was decreased and, because this enzyme is involved in the rate of 5-HT synthesis, may be the reason for reduced brainstem serotonin synthesis. There was also an increase in pineal serotonin levels and synthesis as well as an increase in cholesterol and triglyceride levels. Altered autonomic signaling to pancreatic islets could increase insulin release while suppressing glucagon. This would be ideal for storing calories in triglyceride form, thus lowering triglyceride serum levels. Therefore, because the hypothalamus has been proposed to act as a modulator for autonomic signaling, it may also alter sympathetic nervous system signals to fat pads, resulting in a difference in fat deposition between the O-M and S5B/P1 rat strains (Weekley, Maher, and Kimbrough, 1982).

The difference in the strains’ susceptibility to obesity when fed a high-fat diet may be due to altered rates of norepinephrine (NE) and epinephrine (E) turnover. Norepinephrine concentrations in the adipose tissues were greater in the S5B/P1 rats, regardless of diet type. NE turnover rates were similar across strains, but higher in S5B/P1 rats when both strains were given a high-fat diet. This suggests that S5B/P1 rats have greater sympathetic tissue innervation. The higher NE turnover rate may be due to
increased NE synthesis in neurons. This also suggests that the decrease in sympathetic nervous system activity is reduced by starvation. Cold exposure also elicited an increase in NE turnover. It is important to note that although energy intake was similar across strains, the O-M strain gained more weight than their S5B/P1 counterparts, suggesting that effect of diet composition is greater in S5B/P1 than O-M rats (Fisler, Yoshido, and Bray, 1984).

When exposed to high-fat diets, both strains initially compensated for the increase in caloric density by reducing their intake. However, this response diminished in the O-M rats with the progression of time. This suggests that the palatability of the food and metabolic factors, 3-OHB and GABA, supersede caloric density signals in the O-M strain. 3-OHB may regulate food intake, whereas GABA increases with fat intake. The S5B/P1 rats have elevated levels of ketones and 3-OHB transport across the blood-brain barrier, suggesting a habitual exposure and down-regulation of 3-OHB. The S5B/P1 strain yielded greater blood and brain 3-OHB levels, but the ratio of blood to brain 3-OHB levels did not differ between the strains. This increase in 3-OHB levels correlated with a decrease in food intake. O-M rats had an inverse effect in that the increase in fat intake promoted ketogenesis and 3-OHB concentrations. Hypoglycemic animals experience an increase of GABA in the MH with a decrease in the LH. GABA in the LH is lower in hungry rats and higher in satiated rats. GABA concentrations increased with increasing fat intake, more so in the S5B/P1 rats. When GABA levels were maintained, food intake decreased. This suggests that perhaps a reduced food intake decreases GABA levels, but increased GABA levels inhibit food intake in a dose-dependent manner. Therefore, the S5B/P1 strain may be able to adjust to energy dense diets by
suppressing food intake via elevated brain GABA levels (Fisler, Shimizu, and Bray, 1989).

5-HIAA, a serotonin metabolite, and serotonin levels were lower in the extracellular MH fluid of the O-M rats in comparison to the S5B/P1 strain when given a low-fat diet. S5B/P1 rats also demonstrated lower ratios of MHPG/NE and 5-HIAA/5-HT, suggesting that these pathways are more active in inactivating NE and 5-HT. During the initial days of eating a high-fat diet, serotonin levels rose 100% in O-M rats but decreased by 10% in S5B/P1 rats. This increase in the O-M strain’s serotonin levels was accompanied by a 50% increase in food intake and norepinephrine. Along with an increase in NE, MHPG levels rose slightly with a decrease in the MHPG/NE ratio. NE release was faster than NE metabolism, suggesting an acceleration of NE turnover. Initially, the S5B/P1 strain demonstrated no increase in food intake or NE levels, but 5-HIAA levels as well as the 5-HIAA/5-HT ratio increased in both strains upon eating a high-fat diet. However, four weeks of eating a high-fat diet caused NE turnover in brown adipose tissue to increase by 40% in S5B/P1 rats, whereas no increase was observed in the O-M rats. This suggests that serotonin metabolism is increased through 5-HIAA in both the O-M and S5B/P1 strains, and the increase in the O-M’s ambient serotonin levels is in response to food intake (Shimizu, Fisler, and Bray, 1994).

**Signaling**

NPY is essential in regulating food intake and energy metabolism by acting through the hypothalamus. The Y-5 receptor (Y5-R) is potentially the mediator of NPY-induced feeding, stimulating food intake, with partial influence of the Y-1R, which is involved in decreasing food intake. In comparing the hypothalamic neuropeptide
expression and receptor subtypes between O-M and S5B/P1 rats, O-M rats yielded a higher expression of NPY Y-1 and Y-5 receptors with the 5HT-2c receptor expression being equal among the strains. The increased expression of the receptors is consistent with the O-M’s increased susceptibility to obesity. The O-M’s higher levels of NPY, in spite of their higher leptin levels, support a difference in the regulation of NPY gene transcription between the two strains. Because leptin suppresses NPY activity and increases Y1-R activity, the O-M strain may have a resistance to leptin due to increased leptin levels, NPY activity, and Y1-R activity. Also, the O-M strain demonstrated an increase in NPY and receptor expression, suggesting that they may exhibit an overall increase in the activity of the NPY pathway. Diet had no effect on the receptor expression or on dietary preference. This lead to the conclusion that dietary fat induces increased serotonin activity. The lower receptor levels observed in S5B/P1 rats is consistent with their attenuated response to a high-fat diet. However this loss of response may also be due to enhanced melanocotin or cocaine-amphetamine related transcript (CART) system activity, increased sympathetic nervous system activity, or reduced concentrations of agouti-related protein (AgRP) (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002).

CRH inhibits food intake and antagonizes the stimulatory effects of NPY. CRH antagonists enhance the effects of NPY, thus stimulating food intake. This indicates that there is an equal concentration of CRH and NPY in the PVN, which controls the regulation of food intake (Lin, York, and Bray, 1996). Both strains displayed similar responses to CRH, but O-M rats had increased CRH levels in the hypothalamus.
Increased CRH levels and corticosterone levels imply an alteration in the HPA-axis of O-M rats (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002).

Galanin and β-casomorphin, like NPY, are metabolic neuropeptides involved in feeding behaviors. Although galanin stimulated food intake in O-M rats eating both high- and low-fat diets, S5B/P1 rats demonstrated a decreased response to galanin, suggesting that galanin may increase fat intake. Since the S5B/P1 strain of rats have demonstrated elevated levels of enterostatin, and enterostatin has shown to reverse hyperphagia, then the impaired response observed in S5B/P1 rats may result from inhibitory effects of enterostatin. It has also been proposed that galanin, rather than stimulating fat intake, stimulates the intake of the rats’ preferred macronutrient choice. β-casomorphin interacts with opioid receptors and therefore is influential in regulating fat intake. β-casomorphin antagonizes the anorexic effect of enterostatin and therefore may participate in a regulatory feeding pathway. It may have a preferential effect on fat intake in that it suppressed feeding in O-M rats given a high-fat diet and had no response on the O-M rats given a low-fat diet. Its lack of effect on the S5B/P1 strain supports the hypothesis of a reduced sensitivity to fat-metabolic peptides or an active inhibitory mechanism in their feeding pathway (Lin, York, and Bray, 1996).

3-OHB transport across the blood-brain barrier may be one method for monitoring nutrient signals from fat metabolism. A higher rate of transport may increase CSF 3-OHB concentrations and therefore act as metabolic feedback mechanism. Age and dietary fat intake affects the uptake of 3-OHB into the brain. Uptake increased during the first twenty days of life, then leveled out to adult levels. The transport and uptake of 3-hydroxybutyrate across the blood-brain barrier was greater in the S5B/P1
strain and increased two-fold in both strains when fed a high-fat diet (Bray, Teague, and Lee, 1987). 3-OHB injected into the third ventricle suppressed food intake and reduced body weight in the O-M strain, but not in the S5B/P1 rats, when given high- and low-fat diets. Insulin injections in conjunction with a low-fat diet reduced food intake in both O-M and S5B/P1 rats, but had no effect when given with a high-fat diet. This suggests that S5B/P1 did not respond to the intraventricular infusions of 3-OHB or perhaps their brain is insensitive to insulin when free fatty acid utilization is high (Arase, Fisler, Shargill, York, and Bray, 1988).

Glucose transport was unaffected by diet (Bray, Teague, and Lee, 1987). Chronic infusion of glucose into the third ventricle induced weight loss and temporary hypophagia, followed by a stabilization of body weight (Arase, Fisler, Shargill, York, and Bray, 1988). Since glucose is the primary fuel for the brain, its supply to the brain must be maintained regardless of exogenous or endogenous manipulations (Bray, Teague, and Lee, 1987). However, there were differences between the strains, dependent upon diet, in the transport of ketone bodies. A high-fat diet increased the amount of ketones entering the brain. This suggests that increased ketone transport is the mechanism by which the brain increases ketone metabolism during fasting and high-fat diets (Bray, Teague, and Lee, 1987).

High-fat diets have been shown to diminish the firing rate of sympathetic neurons innervating adipose tissue in O-M rats. RU 486 prevented obesity and promoted the loss of existing fat cells by blocking the type II glucocorticoid receptors, hypothalamic feedback control of CRF secretion, and pituitary ACTH production. This is indicated by the large increase in corticosterone levels in the blood, adrenal hypertrophy, and reduced
activity of hepatic tyrosine aminotransferase. Hepatic tyrosine aminotransferase is an enzyme that is controlled directly by glucocorticoids. RU 486 reversed the obesity induced by a high-fat diet by reducing body and fat pad weight and suppressing food intake. Because RU 486 also acts as a progesterone antagonist, its anti-progesterone receptor activity prevented progesterone from stimulating food intake and promoting fat deposition. Also, RU 486 is more effective in female O-M rats than male O-M rats (Okada, York, and Bray, 1992).

**Summary**

Both strains’ responses to food intake and dietary preference were affected by the manipulations involved in the hypothalamic signaling and neurotransmitter production and release. O-M rats may have an altered serotonin metabolic system that contributed to their susceptibility to obesity (Kimbrough and Weekley, 1984). The differences in the serotonergic neurons and tryptophan metabolism may have contributed to the development of hyperphagia and obesity (Weekley, Maher, and Kimbrough, 1982). There was a greater endogenous norepinephrine concentration and turnover rate in the brown adipose tissue of S5B/P1 rats. This suggests that the difference in the strains’ responses to a particular diet may be due to altered turnover rates of norepinephrine and epinephrine (Fisler, Yoshido, and Bray, 1984). 3-OHB is a potential signal for body weight regulation (Fisler, Shimizu, and Bray, 1989), and subcutaneous, intraventricular, and intracerebroventricular injections of 3-OHB reduced food intake, whereas ventricular infusions decreased nighttime food intake in O-M rats (Arase, Fisler, Shargill, York, and Bray, 1988). The increase in 3-OHB transport across strains eating a high-fat diet, with no difference in glucose transport, suggests that a resistance to obesity is therefore
associated with increased 3-OHB transport across the blood brain barrier (Bray, Teague, and Lee, 1987). Brain GABA increases in S5B/P1 rats with increased fat intake. This increase in brain GABA levels may enable the S5B/P1 strain to adjust to energy dense diets and suppress food intake, suggesting that brain GABA influences the ability to adjust to an energy dense diet through a suppression of food intake (Fisler, Shimizu, and Bray, 1989). The concentrations of neuronal neurotransmitters were identified through examining the changes in concentrations of norepinephrine, serotonin, MHPG, and 5-HIAA in the VMH in relation to eating a high-fat diet. Ambient extracellular monoamines in the MH were found to be lower in O-M rats which also exhibited a greater catecholamine response to dietary fat (Shimizu, Fisler, and Bray, 1994). Dietary fat and genetics affected the expression of hypothalamic genes associated with food intake. Over-activity of the NPY system may be a contributor to the development of obesity in O-M rats and 5HT-2 receptor expression may be altered by dietary fat (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002). The S5B/P1 rats’ regulatory system, activated by galanin and β-casomorphin, may be defective in its control of fat intake (Lin, York, and Bray, 1996). RU 486 and glucocorticoid type II receptors modulate body fat deposition by acting on fat pads. Whereas glucocorticoid receptors are essential to the development of obesity, RU 486 blocks the glucocorticoid receptors, and thus reducing weight gain in O-M rats fed a high-fat diet. However, when given a low-fat diet, RU 486 had no such effects (Okada, York, and Bray, 1992).

**Lactation**
Lactation requires increased energy needs through mobilizing fat stores and increasing food intake. The increase in food intake is directly correlated with increased litter size and increased prolactin levels. Prolactin is a hormone that mediates increases in fat deposition and food intake. When prolactin was injected, food intake was increased in a dose-dependent manner. Also, changes in circulating progesterone and estrogen alter food intake in that progesterone increases food intake and estrogen suppresses food intake. The increase in serum progesterone was accompanied by an increase in prolactin levels (Gerardo-Gettens, Moore, Stern, and Horwitz, 1989).

Gerardo-Gettens et al. (1989) investigated prolactin’s ability to stimulate food intake in the absence of progesterone. Ovariectomies reduced ovarian hormones and thus induced hyperphagia. When estrogen was injected into ovariectomized rats, the estrogen treatment increased serum prolactin levels but decreased food intake. This suggests that prolactin-induced hyperphagia was not dependent upon ovarian progesterone. Prolactin may directly act to stimulate food intake or antagonize estrogen’s suppressive influence on food intake. The increased food intake did not lead to an increase in body weight, but did stimulate an increase in white fat deposition. This indicates that prolactin requires the circulation of both estrogen and progesterone for weight-gain to occur.

Summary

With elevated levels of prolactin, food intake increased independently of progesterone in hyperprolactinemic O-M rats. Therefore, it can be concluded that prolactin stimulates food intake when ovarian progesterone is absent and antagonizes the suppressive effects of estrogen on food intake (Gerardo-Gettens, Moore, Stern, and Horwitz, 1989).
Exercise

Exercise is an important component in combating obesity. Its proposed effects include increases in energy expenditure, decreases in food intake, and decreases in appetite. Its appetite suppressant effect is only found in males, whereas in females, appetite and food intake is increased and a maintenance in body weight (Hoffman-Goetz and MacDonald, 1983). Typical changes associated with chronic exercise include a reduction in body fat, increased muscle capacity, and altered circulation of hormones such as insulin, epinephrine, and glucocorticoids. However, when exercise is terminated, exercise-induced changes are reversed to pre-exercise levels such that there is a rebound effect of body fat and body weight and increased lipid concentrations (Applegate and Stern, 1987). Males and females respond differently to exercise, such that males experience a diminished appetite and reduced body weight and females experience an increase in food intake but maintain their body weight. Much of these differences are due to the type, intensity, and duration of the exercise period (Applegate, Upton, and Stern, 1982).

High-fat fed male rats consumed greater amounts of food than their non-exercised and low-fat fed counterparts. This lack of food suppression in the O-M rats suggests that the difference in food intake was due to the rats’ obesity condition rather than the palatability and composition of the diet. The exercised male O-M rats reduced their weight, but, despite the significant changes in caloric output (Applegate, Upton, and Stern, 1982), did not decrease their food intake. This weight loss in the O-M rats may occur because of an increase in energy expenditure rather than a reduction in food intake.
Males of normal weight reduced their weight gain and food intake in response to increased treadmill exercise. Dietary obesity was not induced in female rats which could reflective of a difference in age, dietary fat intake, and/or impairment in the adipose tissues’ ability to discriminate between macronutrient compositions. When the female O-M rats were exercised, body weight and food intake was maintained independent of dietary food content. This maintenance in body weight may be due to enhanced energy expenditure, energy efficiency, or changes in body composition. Also, the additional output of the male rats may account for the reduced weight gain observed in the male rats as compared to the female rats (Applegate, Upton, and Stern, 1982). Body weight regulation may be diminished in normal weight males in comparison to female rats (Hoffman-Goetz and MacDonald, 1983).

Exercise significantly reduces light cycle food intake. This may account for the reduction in adipose tissue that was paralleled with a decrease in LPL activity. There was also a decrease in insulin levels, but an increase in insulin sensitivity with chronic exercise. This sensitivity is altered through an improvement in glucose tolerance, glucose uptake, or insulin binding. Forty-eight hours post-exercise, insulin levels had returned to those of sedentary rats. Therefore, exercise termination may decrease sympathetic tone and increase insulin levels, thus allowing for glucose uptake in adipocytes and enhancing lipid synthesis. Sixty hours after exercise termination, insulin levels had risen above sedentary levels. This may have resulted from the absence of inhibitory effects on food intake produced by treadmill activity. The increase in food intake also led to increased adipose LPL activity above that found in sedentary rats, eighty-four hours post-exercise. Since LPL activity, in correlation with plasma insulin levels, promotes lipid deposition,
increased activity may be due to increased plasma glucose and insulin levels caused by increased food intake (Applegate and Stern, 1987).

**Summary**

Body weight is less closely regulated in normal weight males than in females. The dietary obesity observed in male rats is similar to that observed in the females’ exercise-induced energy expenditure (Hoffman-Goetz and MacDonald, 1983). Terminating exercise increased lipid deposition at a rapid rate. The increased lipid deposition resulted from an increase in food intake, plasma insulin levels, and enhanced LPL activity. Eighty-four hours post-exercise, changes in hormonal balance occurred, which may have had an effect on food intake, substrate uptake by peripheral tissues, and lipid synthesis and deposition (Applegate and Stern, 1987). Exercise diminished body-fat percentage in both males and females, but increase body-protein percentage only in males. A short term exercise program produced significant changes in the males, whereas females were more resistant to the effects of the exercise regimen (Applegate, Upton, and Stern, 1982).

**Discussion**

Through comparing each strain’s physiological and behavioral characteristics and isolating the mechanisms involved in their susceptibility to diet-induced obesity, scientists are further in attaining a quantifiable measurement of the effects produced by genetics (Schaffhauser, Madiehe, Braymer, Bray, and York, 2002, and Arase, Fisler, Shargill, York, and Bray, 1988). Although a definite cause for obesity has not been identified through the research conducted with O-M and S5B/P1 rats, it has enabled a
better understanding of the importance of genetics on one’s susceptibility to obesity. In addition to behavioral factors, individual variances in sex, metabolic processes, taste preferences, and body composition contribute to the body’s plasticity for fat recognition, fat intake, and hormonal and developmental changes. By continuing to investigate the effects of peripheral ingestive signaling pathways, hypothalamic ingestive pathways, lactation, and exercise on the physiological and behavioral mechanisms involved in each strain’s susceptibility to obesity, researchers are closer to identifying potential physiological and pharmacological treatments for diet-induced obesity (Gilbertson, Lidong, Insook, Burks, and Hansen, 2005).


