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Fasting Serotonin Concentrations and Turnover in High Fat Dietary Obesity Susceptible, Osborne-Mendel and High Fat Dietary Obesity Resistant, S 5B/Pl, Rats Fed a High Carbohydrate or High Fat Diet

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ABSTRACT

FASTING SEROTONIN CONCENTRATIONS AND TURNOVER
IN HIGH FAT DIETARY OBESITY SUSCEPTIBLE, OSBORNE-MENDEL
AND HIGH FAT DIETARY OBESITY RESISTANT,
S 5B/Pl, RATS FED A HIGH CARBOHYDRATE OR HIGH FAT DIET

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The goal of this research was to determine if hypothalamic serotonin concentration or turnover differed between male Osborne-Mendel (obese) and S 5B/Pl (non-obese) rats fed a high (72% of energy) carbohydrate or high (72% of energy) fat diet. Two experiments were performed: one with rats 4 weeks old and fed semi-purified diets ad libitum 3-5 weeks and another with rats weaned at 3 weeks and fed ad libitum 16-18 weeks. Hypothalami were removed, tared, and analyzed for serotonin, 5-hydroxyindoleacetic acid (5HIAA), and total protein and wet tissue weight. No differences were found in 5HIAA concentration or 5HIAA/serotonin ratio in either experiment. Serotonin concentration (mean (s dev)) differed for strain ($p<0.01$) at 7-9 weeks: Osborne-Mendel rats fed high carbohydrate, 577 (153) ng/g, Osborne-Mendel high fat, 534 (44), S 5B/Pl high carbohydrate, 499 (149), and S 5B/Pl high fat 446 (92). At 19-21 weeks, serotonin concentration differed for diet ($p<0.05$) with Osborne Mendel fed a high carbohydrate diet, 901 (392), and S 5B/Pl high carbohydrate, 785 (337), Osborne-Mendel high fat, 521 (149), and S 5B/Pl high fat 708 (310). In conclusion, based on this research, hypothalamic serotonin concentration and turnover are not strongly associated with the high fat dietary obesity of the Osborne-Mendel rat as compared to the S 5B/Pl rat.

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INTRODUCTION

The regulation of appetite is continually interesting to the weight conscious American. Obesity, a risk factor for several diseases, requires a multifaceted treatment. It is widely believed that the neurotransmitter, serotonin, is one factor in appetite regulation, which could at times influence obesity (Blundell, 1984; Blundell, 1977; Green, 1984). A method to control obesity would provide an improved quality of life for the people who feel social ostracism due to their poor body image and an increased lifespan for the morbidly obese.

To investigate the role of serotonin in obesity, the development of an animal model for obesity is an important requirement. In 1970, Schemmel (Schemmel et al., 1970) reported that Osborne-Mendel rats had a mean of 39% body fat after having been fed a high fat diet for 20 weeks whereas S 5B/Pl rats had a mean of 14% body fat after 20 weeks fed a high fat diet. The difference was significant at $p<0.01$.

These two strains, the dietary obesity-susceptible, Osborne-Mendel, and the dietary obesity-resistant, S 5B/Pl, provide a useful tool in the study of obesity. They offer a chance to contrast potential obesity-associated factors, such as serotonin level or turnover, between an obese and a lean animal model. The Osborne-Mendel rat reaches a level of only 13% of body weight as fat after 20 weeks fed grain ration while the S 5B/Pl rat has 9% of body weight as fat when

it is fed a grain ration for 20 weeks (Schemmel et al., 1970). It is possible to attribute the Osborne-Mendel obesity to the high fat diet. Any changes in obesity associated factors could also be related to the high fat diet.

Changes in the concentration or turnover of serotonin in rats fed a high fat diet could be related to obesity. This idea is plausible because research with the neurotransmitter shows that it may be altered by diet (Wurtman, 1986).

If serotonin concentrations are changed by diet, it is possible that this is a long term effect such as induction of an enzyme like tryptophane hydroxylase or a short term effect such as a change in precursor availability. Most research seems to have focused on the effects of diet or drugs on brain serotonin concentrations and/or turnover, despite the fact that almost every researcher reports different baseline values for serotonin, which vary from 0.04 nmol/g (Robinson, 1982) to 11.24 nmol/g (VanLoon et al., 1981) in hypothalamic tissue even when the same strain of rat, Sprague-Dawley, and the same detection system, high performance liquid chromatography with flourometric detection, were used. It seems important to have more information about the baseline conditions before questions on the shorter term effects of diet/food on serotonin metabolism are raised.

The purpose of my research was to evaluate the fasted (12 hr) concentrations and turnover of serotonin in high fat dietary obesity-susceptible Osborne-Mendel rats fed a high fat diet compared to the same strain fed a high carbohydrate diet and compared to the high fat dietary obesity-resistant S 5B-P1 strain fed a high fat or high

carbohydrate diet. Serotonin turnover was estimated by the ratio of the concentrations of the major metabolite of serotinin, 5-hydroxy-indoleacetic acid, to serotonin.

LITERATURE REVIEW

Metabolism

The first part of investigating serotonin in it's role as an appetite regulator is understanding the biochemical pathway it follows from ingestion of dietary precursors to the method of metabolite excretion.

Serotonin is synthesized from the large neutral amino acid, tryptophan (Undenfriend et al., 1956). This is an essential amino acid and must be obtained from the diet. Once tryptophan (Trp) is absorbed by the gastrointestinal tract it enters the blood stream and is available to the various cells of the body.

The beginning point in the synthetic pathway of serotonin is the dietary precursor, tryptophan. After absorption, tryptophan is present in the blood. At this stage, what influences are there on tryptophan and its eventual conversion to serotonin? Along with the level of tryptophan consumed, it is important to know the ratio of tryptophan to the other large neutral amino acids (LNAA) (tyrosine, phenylalanine, leucine, isoleucine, valine and possibly methionine and threonine) and the presence of factors that could change this ratio. Amino acids are transported into the brain, across the blood brain barrier, by one of three systems, depending on whether the amino acid is neutral, acidic, or basic. The LNAA transport system, which transports tryptophan, shows preference for large amino acids (Pardridge, 1985),

of which tryptophan is one. A widely reported influence on the Trp/LNAA ratio is the presence of insulin in the blood (Fernstrom, 1985). Insulin promotes the uptake by non-central nervous system (CNS) cells of other large neutral amino acids (LNAA) preferentially over the uptake of tryptophan (Fernstrom, 1985). Transport of LNAA into the CNS uses both an insulin sensitive and an insulin insensitive mechanism (Pardridge, 1985). Because the Trp/LNAA ratio has been increased without promoting the uptake of other LNAA over tryptophan into the brain more tryptophan is available to the brain.

Once tryptophan is transported across the blood-brain barrier, it is hydroxylated by tryptophan hydroxylase to 5-hydroxytryptophan (5HTP) (Mefford and Barchas, 1980). Five-hydroxytryptophan is then converted to 5-hydroxytryptamine (5HT). Tryptophan hydroxylase is the rate limiting step in the synthesis of 5HT. This enzyme was shown to have a phosphorylation/dephosphorylation regulation system (Fernstrom, 1985) that is influenced by calcium, magnesium, and cyclic AMP (Trulson and Crisp, 1985; Messripour and Clark, 1985). Tryptophan hydroxylase also has a Km value similar to or above the tissue concentration of the amino acid substrate (Lovenberg, 1985). This is an important factor because it implies that 5HTP synthesis and, indirectly, serotonin synthesis will increase if the concentration of tryptophan increases, which is an important point in consideration of the original focus on appetite regulation. After synthesis of 5HTP, by tryptophan hydroxylase, 5HTP is quickly converted to serotonin by L-aromatic amino acid decarboxylase (Mefford and Barchas, 1980). Upon release from the neuron into the synapse, by a process generally

believed to be exocytosis (Wolf et al., 1985), 5-hydroxytryptamine (serotonin) is converted into 5-hydroxyindole acetic acid (5HIAA). The degridation of serotonin is a two step process. Serotonin is first converted to 5-hydroxyindole acetaldehyde. This compound is converted by aldehyde dehydrogenase to 5HIAA. 5HIAA is transported out of the brain by an acid transport system (Ashcroft et al., 1968). It is primarily excreted through the urine. There is also a minor pathway by which serotonin is converted to n-acetyl serotonin by n-acetylase then to melatonin and finally to 6-hydroxy melatonin by hydroxy indole-o-methyl transferase in neural cells (Mefford and Barchas, 1980; Theoharides et al., 1982).

Insulin and Obesity

Abnormalities in insulin response among the obese are a widely recorded phenomenon. Abnormal insulin levels or response have been found in obese humans, rats and monkeys (Jen et al., 1985; Swartz et al., 1985; Axen et al., 1985; Campfield and Smith, 1985; Drewnowski et al., 1985; Stein et al., 1985).

It has been reported that, compared to Osborne-Mendel rats, S 5B/Pl rats have a lower concentration of serum insulin (Yoshida et al., 1986) and a decreased tolerance of exogenous insulin (Schemmel et al., 1982). This information combined with the research on the effect of insulin on serotonin precursor availability, makes differences in serotonin level or turnover, potentially mediated through the action of insulin, an interesting possibility. The possibility of this connection is further supported by the findings of Ashley (Ashley et al., 1985), who

found a decrease in TRP/LNAA ratio in obese humans with hyperinsulinemia. Ashley proposed a decrease in serotonin turnover based on this lower ratio. Smythe (Smythe et al., 1985) investigated the acute effects of insulin on serotonin concentration and found a decrease in hypothalamic serotonin when fed or fasted rats received a bolus dose of insulin ten minutes prior to sacrifice. This research does present some contradiction to Ashley's work, in that Smythe found an increase in turnover, as measured by 5HIAA/serotonin ratio, as a result of insulin, whereas Ashley hypothesized a decrease in serotonin turnover in hyperinsulinemia. There are some questions presented by Smythe's methodology. Smythe used only one time point to measure the effect of insulin and presents no particular rationale for this time point choice. Because insulin levels vary in response to a meal and serotonin levels have been shown to have diurnal variation, it seems important to investigate the question of serotonin concentrations and mechanisms further.

Another factor in understanding this mechanism is to understand the effect of a high fat or high carbohydrate diet on serum insulin concentrations. Inoue (Inoue et al., 1977) found a non-significant reduction in insulin secretion from islet cells of female Charles-River rats fed a 60% fat diet. Blazquez and Quijada (Blazquez and Quijada, 1968) reported a significant ($p<0.01$ decrease in serum insulin when male Wistar rats were fed a high fat, compared to a high carbohydrate diet. Schemmel (Schemmel et al., 1982) found a similar decrease in serum insulin when Osborne-Mendel rats were fed a high fat diet. This information offers the potential for a change in serotonin concentration by a change in a serotonin affector.

Serotonin and Diet

The investigation of serotonin concentrations when an animal is fed a high fat versus a high carbohydrate diet has been very limited. Kimbrough (Kimbrough and Weekley, 1984) compared Sprague-Dawley and Osborne-Mendel rats fed a high fat (20% corn oil) diet to the same strains fed a low fat (5% corn oil) diet. They found no change in brain stem serotonin concentrations in the Osborne-Mendel but did find a significant decrease in brain stem serotonin concentrations in Sprague-Dawley rats fed a high fat diet. Interpretation of this information is complicated by the lack of a precise description of the brain tissue dissected: it is described only as the brain stem. Kimbrough (Kimbrough and Weekley, 1984) does suggest that the Osborne-Mendel rat may show an abnormal brain stem serotonin concentration in response to a low fat, high carbohydrate, diet. While this information could fit the increased insulin tolerance found in the Osborne-Mendel rat (Schemmel et al., 1982), it does not fit the obesity found in Osborne-Mendel rats, which are obese when fed a high fat diet, but maintain a normal percentage of carcass weight as fat when fed a high carbohydrate diet.

Though there has been a significant amount of research into the relationship between diet and serotonin (Fernstrom and Wurtman, 1972; Knott and Curzon, 1974; Ashley and Anderson, 1975; Ashley and Anderson, 1977; Yokogoshi et al., 1986), each of these experiments has altered the protein intake in general or the tryptophan intake specifically rather than making a comparison between a high fat and a high carbohydrate diet. Also, these researchers chose to use Sprague-Dawley or Wistar

rats which are not as well defined as models for dietary obesity (Schemmel et al., 1970).

Pathway Effecting Drugs

In research and treatment of some serotonin-related conditions, a variety of drugs have been used to manipulate the serotonergic pathway within the CNS. The enzyme tryptophan hydroxylase can be inhibited with the drug para-chlorophenylalanine. This drug has been used to deplete brain serotonin by blocking synthesis (Marsden and Curzon, 1976; Sheard and Aghajanian, 1968). Depletion, which can last for weeks or months after one injection (Fuller, 1985), is caused by neurotoxic disintegration of serotonergic neurons. For research purposes a drug called NSD 1050 (3-hyxroxy benzylhydrazine hydrochloride) and another drug referred to as RO 4-4602, have been used to measure the accumulation of 5-hydroxytryptophan in the brain (Kilts et al., 1981; Shannon et al., in press; Herr et al., 1975; Bourgoin et al., 1980; Boadle-Biber et al., 1983). These drugs block the enzyme L-aromatic amino acid decarboxylase and allow for the accumulation of 5-hydroxytryptophan. The rate of 5-hydroxytryptophan synthesis can then be calculated and used to estimate serotonin synthesis. Another point in the pathway where serotonin is manipulated is at the neuron where treatment with the drug reserpine causes release of serotonin from vesicles. Reserpine treatment is generally used to evaluate behavioral changes (Wolf et al., 1985) or for depletion of brain serotonin prior to some other treatment (Lichensteiger et al., 1968). The next point where the pathway has been altered for research purposes

is at the enzyme momoamine oxidase. There are a variety of drugs used in research and therapy which block monoamine oxidase. There are two types of monoamine oxidase, A and B. Some drugs are thought to act preferentially on one type or the other, while other drugs are believed to effect both types of the enzyme more or less equally. Clorgyline inhibits monoamine oxidase A preferentially (Campbell et al., 1979). L-deprenyl seems to show a greater effect on monoamine oxidase B (Johnston, 1968; Knoll and Magyar, 1972; Fowler and Tipton, 1982) than on monoamine oxidase A. Iproniazid, phenprazine (Bliss et al., 1968), tranylcypromine (Neff and Tozer, 1968), and pargyline (Tozer et al., 1966; Yamada et al., 1985; Yarbrough, et al., 1971; Yarbrough et al., 1973) have all been used for nonspecific inhibition of monoamine oxidase. The final area where drugs are used to research the serotonin pathway is blockage of 5HIAA transport from the brain. Probenicid is used to block the exit of 5HIAA from the brain (Yarbrough et al., 1971; Yarbrough et al., 1973; Neff et al., 1967; Werdinius, 1967; Wong, 1985). Accumulation of 5HIAA can then be used to estimate serotonergic activity.

Anorectic Agents

There are two main classes of drugs that work as anorectic agents (Blundell, 1984). One group functions through the catecholaminergic system and is usually represented by the drug amphetamine. Amphetamine is thought to work as an indirect agonist for norepinephrine and dopamine, releasing them to act at the postsynaptic receptors. This anorectic agent does not seem to affect the serotonergic pathway and

therefore will not be discussed in length.

The second group of anorectic drugs works by blocking the uptake of serotonin from brain nerve endings. This is believed to increase the concentration of serotonin in the synaptic cleft (Wong et al., 1985; Geyer et al., 1978). Fluoxetine is a drug that works as a selective inhibitor of serotonin uptake by neurons (Werdinius, 1967; Wong et al., 1985; Geyer et al., 1978; Marsden et al., 1979). In 1976, Goudie (Goudie et al., 1976) reported that fluoxetine decreased solid food consumption in rats and worked synergystically with 5-Hydroxy-tryptophan, the serotonin precursor. The mechanism has yet to be explained. Fenfluramine, another serotonin releasor, has been successfully used to aid obese patients in weight reduction (Strain and Zumoff, 1985). Finally, among the anorectic agents, there is the drug para-chloroamphetamine, which works by promoting release of serotonin by neurons rather than inhibiting uptake. These drugs have been used to confirm the interaction of serotonin and appetite in terms of total calories (Goudie et al., 1976; Breisch et al., 1976; Grinker et al., 1970) or in relation to consumption of protein, carbohydrate, and fat (Wurtman et al., 1985; Wurtman and Wurtman, 1977; Orthen-Gambill and Kanared, 1982). Breisch (Breisch et al., 1976) was able to induce hyperphagia with para-chlorophenylalanine which correlated with the duration of serotonin depletion in the brain. Some of the work done by Wurtman et al. has been carried out with humans (Wurtman et al., 1985). The human response pattern was similar to the response pattern of their rat studies. All of this work has helped to clarify which behaviors may be under serotonin regulation.

Brain Lesions

Lesions of the hypothalamus were shown to markedly deplete forebrain serotonin (Moore and Heller, 1967; Heller and Moore, 1968). Fuxe, in 1965 (Fuxe, 1965) reported that the hypothalamus is heavily innervated with serotonin neurons. Hypophagia has been induced with lesions of the hypothalamus. This information has resulted in a focus on the hypothalamus as a brain region of interest in serotonin research related to appetite regulation.

Methods for Serotonin Turnover Evaluation

There are a variety of means for measuring serotonin turnover as an indicator of neuronal activity. One relatively simple turnover method that has received some use is the ratio of 5HIAA to serotonin. Researchers have reported that electrical stimulation in areas where serotonin neurons are aggregated produces a decrease in serotonin and an increase in 5HIAA (Ahgafanian et al., 1967; Kostowski et al., 1969; Sheard and Zolovick, 1971). Ashcroft (Ashcroft et al., 1968) used this ratio as a way to compare serotonin turnover in various parts of the dog brain. Sparks (Sparks et al., 1985) and Smythe (Smythe et al., 1985) later used this method as a means of estimating turnover of serotonin in rat hypothalamus. This method allows estimation of turnover on an animal-by-animal basis and does not require use of any drugs, such as those mentioned earlier. This simplifies the procedure compared to the use of a monoamine oxidase inhibitor and the slope of a line based on serotonin concentrations over time. The simpler method reduces the possibility of experimental error and the materials cost.

The 5HIAA/serotonin ratio has also been reported to show a better correlation with electrical stimuli to the brain (Shannon et al., in press) than the monoamine oxidase inhibition method developed by Tozer et al (Tozer et al., 1966). In a comparison of four methods of evaluating serotonergic nerve firing, the monoamine oxidase inhibition method, using the drug pargyline, was the only method found to be non-responsive to changes in the intensity of serotonergic nerve stimulation. The 5HIAA/serotonin ratio method was found to be a valid index of neuronal stimulation. A limitation of the 5HIAA/serotonin ratio method is the possibility that both 5HIAA and serotonin may not be responding in the same way at the same time to the same stimuli. If there is question about other influences on 5HIAA and serotonin concentration when the ratio is measured, then the validity of this index of serotonin turnover may be questionable. Despite this problem, the use of the ratio method and the validity of results that have been reported by a number of researchers make it a useful tool for serotonin research (Shannon et al., in press; Smythe et al., 1985; Sparks et al., 1985). The slightly simpler methodology compared to monoamine oxidase inhibition is also an asset.

Though it has been represented as non-responsive to serotonergic neuronal stimulation, as mentioned earlier (Shannon et al., in press), the monoamine oxidase inhibition method, using pargyline, has received wide use (VanLoon et al., 1981; Neckers and Meek, 1976; Li et al., 1984; VanLoon et al., 1981). As a method for turnover estimation it's limitations are, along with the reported problems of validity, the requirement of more than one animal to obtain a turnover value based on

the slope of a line (Tozer et al., 1966), as it requires one animal for each time point on each line. The determination of the most valid line is a compromise between the increased accuracy with an increased number of time points for the line and the increased error due to inter-animal variation. There is also the added cost of animals and drugs.

Another method that has been used is the inhibition of the enzyme L-aromatic amino acid decarboxylase with a resulting accumulation of 5-Hydroxytryptophan (Morot-Gaudry et al., 1984; Johnson and Crowley, 1982; Knott and Curzon, 1974). While it was found a valid indices in comparison with three other methods (Shannon et al., in press) (increased 5HIAA, 5HIAA/5HT ratio, and monoamine oxidase inhibition with pargyline) of evaluating neuronal activity, it is again a method which requires more than one animal to determine a turnover rate, with the complications of inter-animal variation. It also has the complications of added cost and the procedure of injection and the added cost of animals. A final point about this method is that while it seems clear that 5-Hydroxytryptophan is converted directly and rapidly to serotonin, this method does measure a precursor of the neurotransmitter rather than the transmitter itself.

The final method evaluated in the comparison work of Shannon (Shannon et al., in press) was an increase in the level of 5HIAA with stimulation of neurons. This method was found to be a valid index of neuronal activity by Shannon et al., but has also been reported as a less valid index (Trulson and Crisp, 1985). It has been used in evaluation of 5HT turnover (Knott and Curzon, 1974). This method also does not use the neurotransmitter itself, but rather the major metabolite

of the transmitter. This measure could be easily used with 5HIAA/ serotonin ratio because this information would be obtained when the 5HIAA/serotonin ratio data was being collected and could be evaluated with the ratio data.

In summary, there are three methods here that have been presented as valid. Two of those methods are relatively simple and allow estimation with a single animal. They are also closely related in terms of the compound used for turnover estimation. As a beginning either method has much to offer. But, because of the relatedness of these two indices, corroboration with data from L-aromatic amino acid decarboxylase inhibition would strengthen the results.

Serotonin Analysis Procedures

Having determined which compounds to measure with some idea of what tissue would be useful to measure, the next step is choosing the measurement system. Liquid chromatography has been used for a long time in the evaluation of biological compounds. Some of the problems of this method are related to the large volume of eluting solvent required, 0.5-3.0 ml, which results in decreased sensitivity, an increased possibility of sample loss and a relatively long assay time (Mailman and Kilts, 1985; Koch and Kissinger, 1979). Liquid chromatography methods were used in the 1970's (Curxon and Green, 1970; Klien and Weller, 1970) to gain some information about serotonin, but in 1977 Salsa and Blank (Sasa and Blank, 1977) described a new high performance liquid chromatography with electrochemical detection system which has now become the primary means of analysis (Kissinger et al., 1977;

Sperk, 1982; Felice et al., 1982; Picard et al., 1985; Reinhard et al., 1980; Warsh et al., 1979). While methods other than this one have provided good results and continue to do so (Warsh et al., 1980; Wakabayashi et al., 1985), there are some considerations that make high performance chromatography with electrochemical detection very desirable for serotonin research with small amounts of tissue. Gas chromatography with mass spectroscopy has been shown to work well (Warsh et al., 1980), but it is expensive (Wakabayashi et al., 1985) and less sensitive (Oomagari et al., 1984). Radioenzymatic methods are less effective because they cannot measure 5HIAA (Picard et al., 1985; Wakabayashi et al., 1985; Oomagari et al., 1985). High performance liquid chromatography with flourometric detection has been reported as successful (Neff and Tozer, 1968; Mailman and Kilts, 1985; Wakabayashi et al., 1985; Anderson et al., 1982), but it has also been reported as lacking sensitivity (Knott and Curzon, 1974).

High performance liquid chromatography with electrochemical detection requires a very small volume, as little as 20 microliters per injection, and the system works well for oxidizable compounds such as 5HIAA, serotonin and 5-Hydroxytryptophan. It is relatively inexpensive once the system is set up and it is an expedient methodology with assay times of as little as 5 minutes (Lyness, 1982). It is a method that allows monitoring of recovery with correction for each individual recovery. The method is very sensitive. While not the only effective method, it is a very good one. One limitation of this method which should be mentioned is the delicate nature of the instrument. The electrochemical detection allows great sensitivity,

but also requires careful handling.

Summary

Based on the research mentioned here, it seems clear that serotonin is involved in the regulation of appetite and presents some interesting problems for appetite research. The pathway of this compound from intake of dietary precursors to excretion of metabolites is well enough understood to be manipulated in an effort to understand appetite control. There are a number of drugs, such as NSD 1015; pargyline, and probeneicid that have known effects on the various enzymes of this pathway and can be used to help elucidate appetite control. There are also drugs which work as agonists of serotonin receptors, such as fenfluramine, which can be used to study the behavior that serotonin may influence, including appetite regulation. There are a number of methods that have been shown to be valid measures for serotonin turnover; 5HIAA concentration and 5HIAA/serotonin ratio are two of the simpler ones. High performance liquid chromatography with electrochemical detection has been shown to be a very rapid, sensitive, and relatively inexpensive method for the measurement of serotonin, its precursor, and its major metabolite, which are the compounds of interest in the estimation of turnover.

All of the research mentioned here provides a framework in which questions related to obesity, leanness, and appetite control can be investigated.

METHODS

Two experiments were done. The first experiment used rats that were 7 to 9 weeks old when they were killed. These rats were begun on the dietary treatment approximately one week after weaning (approximately four weeks old). Between weaning and the beginning of the dietary treatment all rats were fed grain ration rodent or rodent chow pellets. The first experiment included 35 Osborne-Mendel and 33 S 5B/Pl rats. Seventeen of the Osborne-Mendel rats were fed a high carbohydrate diet and eighteen of the Osborne-Mendel rats were fed a high fat diet. Sixteen of the S 5B/Pl rats were fed a high carbohydrate diet and seventeen of the S 5B/Pl rats were fed a high fat diet. At the beginning of the dietary treatment, the mean weight of the Osborne-Mendel group fed a high carbohydrate diet was 88 grams with a standard deviation of 17 grams; the mean weight of the Osborne-Mendel group fed a high fat diet was 93 grams with a standard deviation of 17 grams. The mean weight of the S 5B/Pl group fed a high carbohydrate diet was 65 grams with a standard deviation of 12 grams and the mean weight of the S 5B/Pl group fed a high fat diet was 64 grams with a standard deviation of 12 grams.

The second experiment used rats that were 19 to 21 weeks old when they were killed. These rats were begun on the dietary treatment at weaning (approximately three weeks old). In the second experiment there

were 32 Osborne-Mendel and 38 S 5B/Pl rats. Ten of the Osborne-Mendel rats were fed a high carbohydrate diet and twenty-two of the Osborne-Mendel rats were fed a high fat diet. Sixteen of the S 5B/Pl rats were fed a high carbohydrate diet and twenty-two of the S 5B/Pl rats were fed a high fat diet. The mean weight of the Osborne-Mendel rats at the beginning of the dietary treatment was 47 grams with a standard deviation of approximately 8 grams. The mean weight of the S 5B/Pl rats at the beginning of the dietary treatment was 36 grams with a standard deviation of approximately 4 grams.

Animals and Housing

The Osborne-Mendel and S 5B/Pl male rats were from the breeding colony of Dr. R. Schemmel (Michigan State University, Department of Food Science and Human Nutrition, East Lansing, MI). They were weaned and placed in individual, suspended, metal cages. The animal room was kept on a twelve hour light and twelve hour dark schedule (06:00-18:00). The room was temperature-controlled to stay at approximately 22 degrees centigrade. At weaning the animals were divided into the four experimental groups described above. Animals were grouped to randomly assign littermates as much as possible and still keep the mean animal weight for each group as near equal as possible. Throughout the experiment rats were weighed once a week between 08:00-09:30 for the first experiment and between 19:00-21:00 for the second experiment with the same balance. All animals were fed from cups. The cups were checked every other day and refilled as needed.

Diet

Throughout the experiment, rats were fed either a high carbohydrate or high fat diet (Table 1). Based on weight, the experimental diets were either 69.75% carbohydrate or 47.70% fat. Per kcal, each diet provided the same amount of vitamins, minerals, and protein. For the carbohydrate diet, the cups held a food saver to prevent the rat from spilling his food. The high fat diet was a solid and was not spilt. Animals received food and water ad libitum until the night before they were killed. Food cups were removed between 21:00 and 22:00. Animals were fasted for twelve hours prior to being killed. They were killed between 09:00 and 11:00.

Group food intakes were estimated at least once prior to killing the animals. Intakes of four animals from each group were recorded for four days, with correction for any spillage, and an average daily intake for each group was calculated. Spilled food was gathered from under the cage and weighed to the nearest gram.

Tissue Collection

Rats were killed by decapitation. The brain was rapidly removed from the skull by cutting along the dorsal surface of the skull from the caudal to the rostral end. The top of the skull was lifted, the brain was removed and then placed on a glass plate over dry ice following the procedure of Warsh (Warsh et al., 1981) with the ventral side up. Dissection of the hypothalamus was based on the method of Holman (Holman et al., 1976). The hypothalamus was isolated by cutting around its perimeter to a depth of 2 mm using a marked scalpel The tissue sample

Table 1. Composition of high carbohydrate and high fat diets.

Ingredients	grams/kg diet	
	High Carbohydrate	High Fat
Casein (1)	200	308
Vitamin mix (2)	10	15
Mineral mix (3)	40	62
Cellulose (4)	20	31
dL-Methionine (5)	3	4
Corn oil	30	-
Cerelose	698	104
Crisco (6)	-	477
% energy from CHO	72%	7%
% energy from fat	7%	72%
Energy per gram* diet (kcal)	3.8	6.0

1 Teklad test diets; high protein casein, 90% protein
2 Teklad test diets; (Association of Official Agricultural Chemists, 1960)
3 Teklad test diets; AIN-76^{tm} (American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies, 1977)
4 Nutritional Biochemical Corp: Alphacel
5 Teklad diets; calc %: c, 40.25%, H, 7.43%, N, 9.39%, O, 21.45%, S, 21.49%.
6 Proctor and Gamble, Cincinnati, Ohio.
* energy was calculated using 4 kcal/g for protein and carbohydrate and 9 kcal/gram for fat

was then lifted from the brain and placed on labeled tin foil on dry ice. The tissue was frozen on the tin foil gently wrapped and then placed in a small plastic sample bag. One bag was used to hold all of the tissue samples collected at one time. Wrapped and frozen, the tissue was held on dry ice until samples were transported to the processing laboratory (approximately 90 minutes). At the laboratory each sample was placed on a new previously tared foil and the wet tissue was weighed to the nearest 0.1 microgram. New foils were used to avoid weighing condensed water from the original foils.

Reagents for Tissue Processing

The mobile phase, in which the tissue was homogenized, was a 50 mM solution with 1.4178 g of monochloroacetic acid, 0.15 g of sodium hydroxide, 40 mg of octyl sodium sulfate, and 0.625 g of EDTA per liter of HPLC grade water. HPLC grade water was obtained from the animal toxicology and physiology laboratory of the Department of Animal Science of Michigan State University. This water was glass-distilled and stored in acid-washed brown glass containers. The mobile phase solution was stirred for one hour with an acid-washed magnetic stirring bar then adjusted to a pH of 3.0 with NaOH and stored in acid-washed brown glass containers under refrigeration. All tissue processing chemicals (sodium bisulfite and n-methyl 5-hydroxytryptamine for internal standard; 5-hydroxytryptamine and 5-hydroxy indole acetic acid for the standard curve) were dissolved in this solution.

If the mobile phase was to be used directly in the HPLC, it was filtered through a 0.22 micrometer nitrocellulose membrane. It was

then degassed under vacuum for approximately one hour. Just prior to use, 13% (vol/vol) acetonitrile was added to the mobile phase.

Processing of Tissue

Processing of tissue was based on the method of Mefford (Mefford et al., 1980). After weighing each tissue sample was placed in a one ml tissue grinder (Thomas Scientific, Swedesboro, NJ), 200 microliters of mobile phase, 100 microliters of 0.5% sodium bisulfite (wt/vol) dissolved in mobile phase, and 100 microliters (50 nanograms) of internal standard, n-Methyl 5-hydroxytryptamine (n-m-5HT) dissolved in mobile phase, was then added and the tissue was homogenized.

Modification of the Mefford method, using mobile phase rather than 1 normal perchloric acid (Mefford et al., 1980), was based on a discussion with Dr. Keith Lookingland (Michigan State University, Department of Pharmacology and Toxicology, unpublished data) concerning the destructive effects of perchloric acid on serotonin and 5-hydroxy-indoleacetic acid. This procedure is further described by Mailman (Mailman and Kilts, 1985).

One ml borosilicate glass tissue grinders were obtained from Radnoti (Thomas Scientific, Swedesboro, NJ). Tissue samples were homogenized while in an ice bath for approximately 60 seconds. Visual inspection was made to ensure that there were no visually detectable tissue residues remaining in the grinder tube. If strands or clumps were present, homogenization was repeated. Tissue samples were held in the ice bath (approximately 0 degree centigrade) during the homogenization process. This procedure is based on the method of Reinhard

(Reinhard et al., 1980). After homogenization, each tissue grinder and pestle was washed down with 100 microliters of mobile phase. The homogenized sample were then transferred by Eppendorf semi-automatic pipette to centrifugation tubes (10 x 75 mm borosilicate glass, VWR Scientific, San Francisco, CA) and centrifuged at 0-4 degrees centigrade and 3079 x g for 10-12 minutes (Sorval ultra centrifuge with a SM 24 head at 5,000 rpm). After centrifugation, the supernatant was removed with a glass pasteur pipette and placed in a microfilter tube with a 0.20 micrometer nitrocellulose membrane (The Anspec Company, Inc., Ann Arbor, MI). The protein precipitate was stored at -70 degrees centigrade until it was analyzed for protein content.

The supernatant in the microfilter tube was then centrifuged for three minutes at 1,500 rpm in an International centrifuge size 2 model V. The filtered supernatant was transferred by glass pasteur pipette to sample tubes (12 x 75 mm sterile capped plastic, Sargent-Welch Scientific, Skokie, IL) and placed on ice until it was assayed for serotonin, 5-hydroxyindoleacetic acid (5HIAA), and n-methyl 5-hydroxy-tryptamine (n-m-5HT), by HPLC-EC. Samples were analyzed within 2 to 4 hours of sample preparation or, after all samples were processed, they were held at -70 C for approximately 18 hours and then analysed by HPLC-EC the next day. Samples run 2-4 hours after processing and then rerun after 18 hours storage at -70 C gave similar values for total serotonin and total 5-hydroxyindoleacetic acid.

Standard Curve

A standard curve was run for the internal standard, n-methyl 5-hydroxytryptamine, serotonin, and 5-hydroxyindoleacetic acid. The standard curve was run at one or two nanoamps full scale and provided a linear response from five to one hundred nanograms injected. Once the linear response pattern had been established, an entire standard curve was not repeated. Prior to each run of tissue samples however, repeated injections of a known amount of each solution were made to establish the detector sensitivity specific for that day. While detector sensitivity would vary from day to day, the relative response to each of the three compounds was constant. This allowed comparison of analyses made on different days based on the relative response to the known amount of internal standard (n-m-5HT).

Recovery

Before the experiments were begun, the recovery of the internal standard was compared to the recovery of serotonin and 5HIAA in tissue samples that had been spiked with a known amount of serotonin and 5HIAA. Serotonin and 5-HIAA were recovered in amounts similar to the recovery of the internal standard. The homogenized tissue solution (without the internal standard added) was divided into two aliquots. To the first aliquot, only a known amount of internal standard was added. The second aliquot was spiked with a known amount of serotonin and 5HIAA, as well as the internal standard. Serotonin and 5HIAA were recovered in amounts from 62% to 111% of the amount added. The internal standard was recovered in amounts from 69% to 116% of the amount added. Recovery of

spiked amounts of the sample were usually in the area of 80-105%.

Analyses for Serotonin and 5-Hydroxyindoleacetic Acid

HPLC-EC analysis was performed with a Brownlee reverse phase column, spheri-5, c-18 chain. A reverse phase spheri-5, c-18 chain guard column was used in front of the analytical cartridge. The electrochemical detection was done with a LC4 amperometric detector (Bioanalytical Systems, West Lafayette, IN) with a glassy carbon electrode set at a potential of +.58 in relation to a Ag/AgCl reference electrode (RE 3 reference electrode, Bioanalytical Systems, West Lafayette, IN). The system used included a Milton Roy model 396-57 minipump, and Altex model 2100 sample injection valve, and a Linear model 1202 recorder for the chromatographs.

Procedures

Sample tissue analysis was done at 2 nanoamps full scale for the detection system. Forty microliters of the filtered supernatant were injected for tissue sample analysis. For each tissue sample two injections were made. The entire group of samples were injected once, then the run was repeated. This serial injection pattern allowed comparison of detector cell response over the period of the run as well as allowing for duplicate measurements of each sample.

Calculations

For each sample, the chromatograph was used to calculate recovery of the internal standard. Based on the peak height of a known amount of

n-methyl serotonin, the amount of n-m-serotonin in the sample injection was calculated and a percent recovery for the sample was determined. The amount of serotonin and 5-hydroxyindoleacetic acid could also be calculated based on the peak height of a known amount of each compound to determine the amount in the sample plus correction for the percent recovery (i.e., based on comparison of peak heights it could be determined that 98% of the internal standard was recovered, that there was one ng of serotonin in the injection of 40 microliters and 12.5 ng of serotonin in the entire sample of 500 microliters (40/500), and that 12.5/.98 = 12.755 the total amount of serotonin in the sample.) The total value was then assessed by comparison of the weight of each hypothalamus to the total value for each compound in each sample (i.e. total ng/hypothalamic wet weight = ng/one gram tissue).

Protein Analysis

The amount of protein in each sample was determined by the method of Lowry (Lowry et al., 1951).

Protein Analysis Reagents

The first reagent was a sodium hydroxide solution used to dissolve the protein precipitate. Forty grams of NaOH were dissolved in one liter of double deionized water in a volumetric flask. Two one ml aliquots of each hypothalamus precipitate-NaOH solution were removed from each sample and placed in a labeled test tubes.

A solution of 2% sodium carbonate and 0.02% sodium tartrate in double deionized water and a solution of 0.5% cupric sulfate in double

deionized water were prepared once and used for all analyses. On each day of analysis, fifty parts of the sodium carbonate-sodium tartrate solution were combined with one part of the cupric sulfate solution to make a fresh solution. Five ml of this solution were added to each 1 ml sample aliquot and standard curve tube, then vortexed and allowed to stand for 20 min.

A solution was made with Folin-Ciocaiteau reagent diluted to half strength with double deionized water. After the 20 min reaction, 0.5 ml of the Folin-Ciocaiteau solution was added to each of the above sample aliquots and standard curve tubes. Each was vortexed as the Folin-Ciocaiteau solution was added and then the tube was allowed to stand for 30 min. before reading absorbance.

Standard Curve for Protein Analysis

A standard curve was run using bovine serum albumin as the reference protein in concentrations of 0, 100, 150, 250, and 350 micrograms albumin per ml double deionized water. Concentrate bovine serum albumin was made with 50 milligrams of crystalline bovine serum albumin dissolved in ten ml of double deionized water. A bovine serum albumin working standard was made by diluting one ml of concentrated bovine serum albumin with 9 ml of 1 molar NaOH on the day of use. It was determined that spectrophotometer readings at 740 mu gave a linear response in the range of dilutions used and that the sample values fell within the linear range. All standards were run in duplicate. The value used was an average of the duplicates. Variation between duplicates averaged ((difference/mean) x 100) 4.67%.

Procedure

The hypothalamic tissue precipitate was placed in 10 ml of 1 molar NaOH. It was then vortexed and two one ml aliquots were removed. Five ml of one solution containing sodium carbonate, sodium tartrate, and cupric sulfate were added to each of the samples with a repipet (Labindustries, Berkeley, CA). This mixture was rapidly vortexed and allowed to stand for 20 minutes. The Folin-Ciocaiteau solution was then added; the mixture was rapidly vortexed and left to stand for 30 minutes. The absorbance of the final mixture was determined by a Beckman DU spectrophotometer at 740 mu. A comparison similar to the one for wet tissue weight of each of the samples was done comparing the amount of protein of each hypothalamus to the total amount of either serotonin or 5 HIAA per gram hypothalamus.

Statistical Analysis

Statistical analysis of the effects of strain, diet, and interaction for each experiment was done by two-way analysis of variance (Gill, 1978). When significant differences were found between variables, statistical analysis of individual means was done by the use of the modified Tukey's honestly significant difference (HSD) test that utilizes a t-like statistic (Gill, 1978). Comparison between experiments was not done because each experiment was run separately and uncontrolled factors made statistical evaluation impossible. Analysis of variance was done on serotonin, 5HIAA, 5HIAA/serotonin ratio, live weight, absolute hypothalmic weight, and absolute hypothalmic protein. Tukey's HDS test was done on serotonin, 5HIAA, and 5HIAA/serotonin ratio, live

weight at sacrifice, absolute hypothalamic wet tissue weight, and absolute hypothalamic protein.

Two-way ANOVA was used to evaluate results because of its greater power. When a difference was found by two-way ANOVA, Tukey's HSD test modified for unequal groups was run for a more precise inspection of the results. At times, the modifications needed to create equal groups for two-way ANOVA made Tukey's HSD test important in the confirmation of significance.

RESULTS

Rats Killed at 7-9 Weeks Old

Average energy intake was calculated for four rats from each treatment group. For strain, in the animals sacrificed at 7-9 weeks old, the average energy intake was significantly different at $p<0.01$ (Table 2). The relevance of this information is better understood by consideration of two other parameters related to animal obesity, weaning live weight and sacrifice live weight. At the beginning of the dietary treatment live weight was significantly greater for the Osborne-Mendel strain and remained significantly greater through the time of sacrifice (Table 3).

Between diets, there was not a significant difference in energy intake in either strain at six weeks old (Table 2). The live weights at weaning were not significantly different between diets but at sacrifice there was a significantly greater weight for the Osborne-Mendel rats fed a high carbohydrate diet to the same strain fed a high fat diet.

Despite the differences in live weight at sacrifice, there were no significant differences in hypothalmic weight or protein in rats sacrificed at 7-9 weeks old. The Osborne-Mendel rat, though significantly larger than the S 5B/Pl rat at weaning and sacrifice, did not have a significantly larger hypothalamus.

Table 2. Mean food energy (kcal) consumed per day during the sixth week by Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

	Osborne-Mendel[a]		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
	98 ± 9*	89 ± 5	75 ± 7	68 ± 5
n	4**	4	4	4

a) Significant difference between strains $p<0.01$
* Mean ± standard deviation
** Each n value based on the mean of four days intake

Table 3. Mean live weights (g) at initiation of experiment (4 weeks) and sacrifice (7-9 weeks) for Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

	Osborne-Mendel		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
n	17	18	16	17
Initial weight (g)	88 ± 17*[b]	93 ± 17[b]	65 ± 12	64 ± 12
Sacrifice weight (g)	247 ± 39[ab]	279 ± 36[a]	175 ± 20[b]	181 ± 31
Hypothalamus weight (mg)	28 ± 3	28 ± 3	28 ± 6	25 ± 4
Hypothalamus protein (mg)	1.8 ± 0.3	1.9 ± 0.3	1.8 ± 0.4	1.7 ± 0.2

* Mean ± standard deviation

a) Difference between strains fed the same diet was significant at $p<0.01$

b) Difference between diets in the same strain was significant at $p<0.05$

The concentrations of neurotransmitters were measured both as nanograms/gram wet tissue and as nanograms per miligram protein. Serotonin concentration was significantly greater in rats fed the high carbohydrate diet when measured as ng/g wet tissue but when measured as ng/mg protein the difference was no longer significant (Table 4). 5HIAA concentration was not significantly different either when ng/g tissue were used or when ng/mg protein were used to evaluate the 7-9 week old rats. 5HIAA concentration is considered an index of serotonin turnover and therefore these results do not support the hypothesis that serotonin turnover is different between these strains or diets. The primary index of serotonin turnover in this work was the ratio of 5HIAA to serotonin. The ratio of the groups were not significantly different which like its associated measure, 5HIAA concentration, does not support a difference in serotonin turnover between strains.

Rats Killed at 19-21 Weeks Old

The average energy intake of the rats sacrificed at 19-21 weeks old was calculated 4 times (Table 5). There was a significant difference in intake between strains at 5, 7, and 8 weeks old. At 19 weeks old, when animals were sacrificed, the intake was significantly different between diets but not strains. This was primarily a result of differences in intake between diets for the S 5B/Pl strain. These differences were significant at p 0.01. The difference in intake associated with strain becomes less of an indication of overeating when the size difference at weaning is considered. Weaning weights were significantly different between strains (Table 6) and remained significantly different at

Table 4. Mean concentrations of serotonin and 5HIAA per gram hypothalamic wet tissue and per milligram protein and ratio 5HIAA/serotonin in 7-9 week old Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

	Osborne-Mendel		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
n	17	18	16	17
Serotonin/[a] hypothalamus (ng/g)	577 ± 153*	534 ± 44	499 ± 149	446 ± 92
Serotonin/ protein (ng/mg)	9.03 ± 2.87	8.24 ± 2.73	7.93 ± 2.54	6.84 ± 1.89
5HIAA/ hypothalamus (ng/g)	488 ± 108	458 ± 87	522 ± 72	493 ± 130
5HIAA/protein (ng/mg)	7.66 ± 2.11	7.01 ± 1.75	8.24 ± 1.59	7.58 ± 2.83
RATIO 5HIAA/5HT	0.87 ± 0.23	0.90 ± 0.24	1.11 ± 0.29	1.17 ± 0.44

a) Difference between diets was significant $p<0.01$
* Mean ± standard deviation

Table 5. Mean food energy (kcal) consumed per day at various ages by Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

Age (weeks)	Osborne-Mendel		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
6[a]	67 ± 6*	65 ± 6	53 ± 4	61 ± 8
n	8**	8	8	8
7[b]	76 ± 6	81 ± 3	60 ± 4	87 ± 8
n	8	8	8	8
8[a]	75 ± 7	74 ± 5	62 ± 8	70 ± 6
n	8	8	8	8
19[c]	35 ± 5	41 ± 6	35 ± 3[d]	67 ± 5
n	4	4	4	4

a) Significant difference between strains for all rats $p<0.01$
b) Significant difference between strains for all rats $p<0.05$
c) Significant difference between diets for all rats $p<0.01$
d) Significant difference between diets in S 5B/Pl rats $p<0.01$
* Mean ± standard deviation
** Each n value was obtained as the mean of four days intake

Table 6. Mean live weight (g) at initiation of experiment (3 weeks) and mean live wt (g), hypothalamus wt (mg) and hypothalamus protein (mg) at sacrifice (19-21 weeks) for Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

	Osborne-Mendel		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
n	10	22	16	22
Weaning[a] weight (g)	47 ± 7*	47 ± 9	36 ± 4	36 ± 4
Body weight[bc] (g)	368 ± 23	471 ± 56	325 ± 45	363 ± 28
Hypothalamus[d] wet tissue (mg)	23.4 ± 4.3	25.4 ± 3.4	25.0 ± 5.4	28.3 ± 7.8
Hypothalamus[c] protein (mg)	2.46 ± 0.29	2.84 ± 0.31	2.21 ± 0.66	2.89 ± 0.89

a) Significant difference between strains for all rats $p<0.05$
b) Significant difference between strains for all rats $p<0.01$
c) Significant difference between diets for all rats $p<0.01$
d) Significant difference between diets for all rats $p<0.05$
* Mean ± standard deviation

sacrifice. By the time of sacrifice, there was also a significant difference in live weight between diets. These differences were focused around the greater weight of the Osborne-Mendel fat fed rat. This group had a mean weight which was significantly greater than the mean weight of the Osborne-Mendel rats fed high carbohydrate or the S 5B/Pl rats fed a high fat diet (Table 6).

The hypothalamic weights were significantly higher in the fat fed groups, which was the same pattern as the live weights, but the largest mean weight was in the S 5B/Pl fat fed group, which were the lighter of the two fat fed groups. Hypothalamic protein followed the pattern of hypothalamic weight and was greater in the fat fed groups and greatest in the S 5B/Pl fat fed rats though the difference between strains was not significant.

Serotonin concentration was significantly greater for rats fed the carbohydrate diet at 19-21 weeks old (Table 7) when the concentration was measured as ng/g wet tissue and significantly greater for the carbohydrate diet in the Osborne-Mendel strain alone when measured as ng/mg protein. There were no other significant differences between groups for serotonin concentration, 5HIAA concentration or ratio in the 19-21 week old age groups (Table 7).

Table 7. Mean concentrations of serotonin and 5HIAA and their ratio per gram wet tissue and per milligram protein in 19-21 week old Osborne-Mendel and S 5B/Pl rats fed a high carbohydrate or high fat diet.

	Osborne-Mendel		S 5B/Pl	
	Carbohydrate	Fat	Carbohydrate	Fat
n	10	22	16	22
Serotonin/[a] hypothalamus (ng/g)	988 ± 238*	521 ± 149	785 ± 337	708 ± 310
Serotonin/[a] protein (ng/mg)	15.1 ± 3.5	8.4 ± 3.6	11.8 ± 4.9	8.9 ± 5.1
5HIAA/ hypothalamus (ng/g)	827 ± 267	773 ± 402	1006 ± 405	795 ± 244
5HIAA/ protein (ng/mg)	8.2 ± 2.7	7.0 ± 4.0	11.4 ± 2.4	8.7 ± 3.8
RATIO 5HIAA/5HT	0.99 0.28	1.63 1.09	1.49 0.78	1.26 0.50

a) Significant difference between diets $p<0.01$
* Mean ± standard deviation

DISCUSSION

Food intake is one measure of a dietary induced obesity. Because it may be an index of appetite, it is a measure that is of special interest to the hypothesis of an altered serotonin concentration or turnover in the rats studied here. If serotonin were a significant factor in the high fat dietary obesity observed in the Osborne-Mendel rat, then alterated intake when the rat was on the obesity-inducing diet would be expected.

Earlier work (Custer, 1983) found a higher energy intake by Osborne-Mendel rats when Osborne-Mendel and S 5B/Pl rats were fed a high fat diet during two hour or twenty minute meal periods. In this earlier experiment (Custer, 1983), total energy intake comparisons were not reported. Thiel et al. (Thiel et al., 1972) reported that Osborne-Mendel rats consumed more high fat diet on a daily basis than S 5B/Pl rats. The similar energy intake between diets reported in this work does not agree with these eralier results. Schemmel et al. (1982) evaluated total energy intake by Osborne-Mendel rats. They found that cumulative energy intake over thirteen weeks was not different between a high fat and a high carbohydrate diet. These results support the results reported here.

In the work reported here, intakes were based on 4 days intake done in the week prior to sacrifice. This is a consideration because

occasionally animals were able to dump their carbohydrate cups. The fat diet did not spill. Spilled food could have two different effects. If food were unavailable prior to doing food intakes, rats would overeat and compensate for lack of food prior to the period when the intake was done. If it were unavailable to the rat, carbohydrate intake values would drop. If spilled diet were not all collected, carbohydrate intake would be artificially high. The collection of spilled diet in this research was done carefully to regain all diet. Therefore, it seems more probable that the carbohydrate intakes are minimum values resulting from possible temporary food deprivation. If this were the case, actual intakes might be greater which would probably not change the statistical difference. Because the value was a mean of four days, the animals who might have dumped their cup on day one or two had time later to catch up their energy intake. This would decrease the effect of spilled diet. There are limitations on this data that make replication very desirable but the results still offer some insight into the mechanism of the Osborne-Mendel rat model of dietary obesity. Based on this work, the Osborne-Mendel obesity does not seem associated with greater energy intake.

At the beginning of the dietary treatment, there was a significant difference in the live weight of the strains. The greater weight of the Osborne-Mendel rat implies that the Osborne-Mendel is a larger rat. A larger rat could consume more diet while maintaining the same percentage of body fat. Between diets, for each strain, the weight was not significantly different. Animals of the same size could be expected to become more obese when consuming more energy. Assessment

of obesity by live weight between diets is therefore possible. Based on this information, weight differences between strains were not considered clear indicators of obesity. If body compositions, percent lean body mass and percent body fat, had been done, it would have been possible to more clearly evaluate the relative obesity of these two strains. Another measure of obesity would have been the removal of one or more fat pads from the carcass at sacrifice. It would have been possible to analyze the size of the fat pad both in absolute weight and as a percentage of the live weight. Differences in weight between diets could be used to evaluate obesity because the strains were comparable in weight at weaning. Work done by Harris et al. with these strains of rats indicated that animals from the same strains had similar tissue protein content even though they were fed different diets (Harris et al., 1977).

It has been demonstrated that (Schemmel et al., 1970; Schemmel et al., 1982) Osborne-Mendel rats fed a high fat diet are heavier than Osborne-Mendel rats fed a high carbohydrate diet. This difference in live weight supports the idea of obesity between diets for this strain. At 7-9 weeks old, the rats used in this work had a significant difference in live weight for diet and strain. These data agree with the work of Schemmel et al. (1970) for the Osborne-Mendel rats and support the use of this animal model in the study of high fat dietary obesity. These results do not agree with earlier studies of the S 5B/Pl rat (Schemmel et al., 1970). Because the earlier work was done with a diet which had a higher percentage of fat, it is possible that the S 5B/Pl rat did not respond in the same way to this diet as it did to the earlier diet. Harris

et al. used these strains of rats fed similar diets and reported that the S 5B/Pl rats weighed the same regardless of diet fed (Harris et al., 1977). Fat pad weights, fat cell size and number have also been reported as similar (Obst et al., 1981). These results do not support the idea of a different response in the S 5B/Pl rat to different levels of dietary fat. These results suggest a need for more work with the S 5B/Pl rat as a model for high fat dietary obesity resistance.

Hypothalamic wet tissue weights at 7-9 weeks were not significantly different for strain or diet. This does not agree with the work of Custer (Custer, 1983) where Osborne-Mendel rats were found to have greater absolute hypothalamic weights than S 5B/Pl rats. Because the method for removal of the hypothalamus is not as exact as would be desired, it is hard to draw any conclusions from this information. Work by Stone et al. (Stone et al., 1981) found similar absolute cerebellar weights in these strains fed a semi-purified 43.75% fat (w/w) or semi-purified high carbohydrate diet. However, cerebrum weight was significantly ($p<0.01$) greater in S 5B/Pl rats and, when expressed as weight/100 grams of body weight, both cerebrum and cerebellum were greater in the S 5B/Pl rat. The relationship between cerebellar or cerebrum weight and hypothalamic weight in these rats has not been investigated, therefore this information offers no specific conflict with or support for the results reported here. The results of Stone et al. were in conflict with the results of Custer, who reported greater hypothalamic weights in the Osborne-Mendel rats. Because the S 5B/Pl in this study were lighter, the results reported here would tend to agree with the work of Stone et al. (Stone et al., 1981) which

reported more brain tissue per 100 grams of body weight in S 5B/Pl than Osborne-Mendel rats.

The hypothalamic protein content at 7-9 weeks old for all groups was similar. Stone et al. (Stone et al., 1981) found similar cerebellar protein content in their comparison of these strains of rats fed a semi-purified high fat or semi-purified high carbohydrate diet but reported a higher absolute protein content in cerebrums of S 5B/Pl rats. The relationship between cerebellar protein and hypothalamic protein has not been investigated in these strains of rat, therefore the comparison must be tentative. The results here do not support the involvement of hypothalamic protein content in the high fat dietary obesity observed in Osborne Mendel rats, in that, while live weights are different between Osborne-Mendel and S 5B/Pl rats, hypothalamic protein content is not. These results have not been analyzed on the basis of protein per 100 grams of body weight. With this type of comparison they may present a different picture, in that the high fat fed animals of the Osborne-Mendel strain are significantly heavier and would therefore have less protein on a per 100 gram body weight basis.

At 7-9 weeks old, the serotonin concentrations were significantly higher in Osborne-Mendel rats compared to S 5B/Pl rats when analyzed by two way ANOVA. This is in agreement with the hypothesis of different serotonin concentrations in the hypothalamus of S 5B/Pl rats. If these results are not spurious, they imply a very complicated mechanism of feeding regulation, because serotonin is a widely reported appetite suppressant and the OM rat has a repeated history of high fat dietary obesity which, if not due to greater metabolic efficiency on

the high fat diet, implies poorer appetite regulation on this diet. A higher concentration of the reported appetite suppressing neurotransmitter, serotonin, is difficult to reconcile with the poorer intake regulation which has been reported in the Osborne-Mendel rats. ANOVA results, which show a significant difference in hypothalamic serotonin concentration between strains, disagree with the results of Custer (Custer, 1983) in which zero time serotonin concentrations for rats six weeks old were similar for each strain fed a high fat diet. It is possible that Custer's results were influenced by the large standard deviation he reported. A larger standard deviation would make the difference between means necessary for significance greater.

The considerable difference in serotonin concentrations reported here vs those reported by Custer present a problem when comparing results, particularly because the animals were from the same breeding colony and the tissue was assayed in the same lab. It is possible that Custer had more of a deeper nucleus, with a high serotonin concentration, in his samples. Custer reported a much higher concentration of serotonin, but cause of the difference is hard to locate because the same method was used with animals from the same breeding colony.

Kimbrough and Weekley (Kimbrough and Weekley, 1984) reported higher serotonin concentrations in Sprague-Dalwey rat brainstems as compared to Osborne-Mendel rat brainstems on a moderate (20%) or low (5%) fat diet. Because the exact tissue analyzed by Kimbrough et al. was not the same, it is uncertain how well Kimbrough's values compare with the results reported here. Another factor is the fed condition, the rats of Kimbrough et al. were allowed access to food up until four

hours before sacrifice while the animals in this study were fasted 12-14 hours prior to sacrifice. Fasting has been reported to increase brain serotonin concentrations in some studies (Loullis et al., 1979; Curzon et al., 1972) and to increase serotonin turnover in other work (Perez-Cruet et al., 1972). The lack of agreement between Custer, Kimbrough et al., and the work reported here makes support for any of the results weak.

The 5HIAA concentrations were determined primarily to calculate the ratio of 5HIAA/serotonin but, because 5HIAA is considered an index of serotonin turnover in some situations (Shannon et al., in press), the levels of 5HIAA were analyzed statistically. Similar concentrations of 5HIAA were found in rats 7-9 weeks old. This does not support a hypothesis of chronically different turnover rates due to strain or diet.

The ratio of 5HIAA/serotonin, which was primary method of evaluating turnover, did not show a significant difference between strains or diets of rats 7-9 weeks old at sacrifice. Again, this does not support a hypothesis of different turnover rates, in a steady state condition.

This disagrees with the results of Custer, which indicated a higher rate of turnover in fasted S 5B/Pl rats compared to Osborne-Mendel rats on a high fat diet. The difference in results may be due to the difference in methodology used. Custer used the MAO inhibitor, pargyline, to block the degradation of serotonin and then assayed the tissue for concentrations of serotonin and 5HIAA over time. In this work, the ratio of 5HIAA to serotonin was done at one time point.

5HIAA/serotonin ratios calculated from the work of Kimbrough et al. show an opposite trend to those found in Custer's work. Their work indicates that the Osborne-Mendel rat has greater hypothalamic serotonin turnover than the Sprague-Dawley rat. Osborne-Mendel rats had a 5HIAA/serotonin ratio 6.5 times the ratio of Sprague-Dawley rats with both strains fed a high carbohydrate (5% corn oil) diet and 3.1 times the ratio of Sprague-Dawley rats with both strain fed a moderate fat (20% corn oil) diet. The Sprague-Dawley rat's ratio was higher on the moderate fat diet compared to the same strain on a low fat diet. The Osborne-Mendel rat's ratio was lower on a moderate fat diet compared to the same strain on a high carbohydrate diet. The indecisive results with the results reported here suggest the need for more research. Until there is some agreement between unrelated research groups, it is very hard to say what is occurring.

The ratios of 5HIAA to serotonin in the work from the laboratory of Kimbrough et al. also prompt the question of how the strains studied here would respond to a high fat or high carbohydrate diet if they had been in a different fed (i.e. 12 hr fasted) state. Looking at serotonin concentration and turnover in various conditions seems critical. It has been reported that rat pineal serotonin turnover varied diurnally (Curzon et al., 1972). This would make the time of day that the animals were killed important as well.

The intake patterns of rats sacrificed at 19-21 weeks changed with age. Early in the experiment the rats had similar intakes between diets but differences between strains. This did not agree with the work of Thiel et al., but again the difference in diets may

have contributed to the difference in response. In the last intake measured in this work the animals were showing a significant difference in intake between diets. Though this agrees with the work of Thiel et al., closer scrutiny indicates a discrepancy. In this work the S 5B/Pl rats consumed more Kcal than the Osborne-Mendel rats when both strains were fed a high fat diet. This information is not only in conflict with the results of related work (Thiel et al., 1981), it also is not supported by the live weights of the animals. Despite the much greater energy intake, the S 5B/Pl rat was still lighter than the Osborne-Mendel rat. The lack of supporting information within this research or outside of it makes it impossible to hypothesize a theory for the differences in weight and intake at the same time. Replication or outside corroboration are necessary to establish the validity of these results.

In the groups sacrificed at 19-21 weeks old, the weights at the beginning of dietary treatment were closer between strains but still significantly different. These weights support the idea that the Osborne-Mendel rat is a larger animal and obesity between strains can not be evaluated on live weight basis. Though the Osborne-Mendel rat has been reported as a more obese animal and comparisons between strains on the same diet are reported, they should be considered in light of the differences in animal size. As mentioned earlier, collection of body composition data or weight of one or more fat pads would have helped evaluation of obesity.

The live weight of rats sacrificed at 19-21 weeks old remained significantly different for diet. These results are supported by the

work of Schemmel et al. (1970) with relation to the Osborne-Mendel rats. They disagree with that work in relation to the S 5B/Pl rats. The sacrifice weights reported here support the idea that either strain of rat will become obese when fed a high fat diet.

The absolute hypothalamic wet tissue weight at 19-21 weeks was significantly higher for S 5B/Pl rats. This is the reverse of the results reported by Custer (Custer, 1983), but shows a pattern similar to the work of Stone et al. (Stone et al., 1981). Again it bears consideration that the method of dissection is not well defined. Results are also complicated by the difference in hypothalamic weight reported by Custer and the results reported here. Custer reported mean weights of 50.4 and 47.1 mg wet tissue for Osborne-Mendel and S 5B/Pl rats respectively. The rats in this experiment had mean weights of 25.4 and 28.3 mg wet tissue for Osborne-Mendel and S 5B/Pl rats, respectively. Custer may have cut deeper when dissecting out hypothalami. This possibility agrees with the possibility that he removed more of a serotonin rich nuclei. All of the values mentioned here were for animals fed a high fat diet. Custer's work was only with high fat fed animals. Therefore comparison to the high carbohydrate fed animals is impossible. Due to the imprecise methodology, these hypothalamic weights offer only limited information about differences between these strains on these diets.

Like the wet tissue weights, the hypothalamic protein content of 19-21 week old rats was significantly different, but in this case the differences were between diets. The protein content was greater for Osborne-Mendel rats fed the fat diet. Because hypothalamic comparison

has not been made in any other studies known to this author at present, there is no work with which to make direct comparisons. Stone et al. (Stone et al., 1981) reported that the protein content of the cerebellum and cerebrum were the same ($p<0.01$) in either of these strains fed either a 40% fat or a semi-purified control diet. The information of Stone et al. does not support the results reported here, but the composition of one part of the brain does not necessarily imply the same composition in another part of the brain. Because the high fat fed rats were eating more Kcal when 19 weeks of age, and the diets were isonitrogenous, the fat fed rats would have been consuming approximately 0.5 extra grams of protein/day. Relevance of this information is limited because the development of the brain occurs early in the animals life while the consumption difference was present only in the end of the treatment period. It is possible that the high fat diet has an effect on hypothalamic protein but related work for comparison is necessary for the development of a clearer picture.

At 19-21 weeks, the hypothalamic serotonin concentration between Osborne-Mendel and S 5B/Pl strains was significantly higher in rats fed a high carbohydrate diet when analyzed by ANOVA but not Tukey's HSD test. ANOVA data agree with the finding of Kimbrough for the brainstem of Sprague-Dawley rats but disagree with their findings for Osborne-Mendel rats. It is possible that this difference is due to the difference in age, sex, and/or diet of the animals. Kimbrough et al. does not report a specific age, but rather describes his animals as 150-200 gram rats. Because our rats were born in the animal room, their exact age could be determined. Age (12-16 weeks compared to 100-106 weeks) had

been reported to increase serotonin concentrations in one study (Sparks et al., 1985), but it is another vague area. Because research in this area has been conflicting, comparisons without consideration of age are hard to support. Kimbrough et al. used female rats. The animals in this research were male. Gender effects on serotonin have not been researched and therefore remain an open question. The high fat diet used by Kimbrough et al. was 20% fat whereas the high fat diet used here was 47% fat (w/w). The high fat diet used in this work had more than twice as much fat by weight while the high carbohydrate diets were nearly the same. This might well have had an effect on serotonin concentrations. Increased serotonin levels on a high carbohydrate diet are a reasonable result in consideration of the effects of carbohydrate on insulin and the effects of insulin on LNAA transport (Wurtman, 1986). Because the younger animals in these studies do not show the same relationship of serotonin concentration to strain as the older animals, it is harder to interpret the data and further investigation seems necessary.

At 19-21 weeks old, the 5HIAA concentration and 5HIAA/serotonin ratio continued to be similar in each strain fed either diet. These results, like the results with 7-9 week old rats, do not support a difference in serotonin turnover in steady state between the obese Osborne-Mendel or lean S 5B/Pl rats fed a high carbohydrate or a high fat diet. The research reported here consistently presents a similar serotonin turnover by either index. Though related research disagrees with this work, it does not form a cohesive enough picture to provide a consistent argument against these results.

Because the two age groups were not designed to be compared, age related comparisons are not directly possible. Still they are an interesting consideration in light of the increased tendency toward obesity in the Osborne-Mendel rat with age (Schemmel et al., 1970). Review of the two groups of data indicates a very noticeable increase in serotonin and 5HIAA concentrations and their ratio with age. Sparks et al. (Sparks et al., 1985) found age related increases in 5HIAA and 5HIAA/ serotonin ratio with age in the rostral and caudal hypothalamus of F-344 rats. Sparks et al. reported changes which were much less than the results reported here. These researchers also report a trend toward decreased serotonin concentrations with age. This is the reverse of what happened in these experiments. Because there is no broad support in the literature for either position, inference is not possible.

The mean ratio of 5HIAA/serotonin in the younger rats of Sparks et al. was comparable to the mean ratios reported in this work, while the ratio for the older rats was greater. Their change in ratio seemed primarily due to a decrease in serotonin with age. As mentioned earlier this did not occur in the work reported here. Their younger rats were 12-16 weeks old at sacrifice while older rats were 100-106 weeks old at sacrifice. This makes their younger rats intermediate in age compared to the two ages used in these experiments while their older rats were approximately five times the age of the oldest rats used in this research. The smaller changes in serotonin, 5HIAA, and ratio reported by Sparks et al. may be reflective of the point in the life cycle of the animals used in these experiments.

While many of the results of this research have some support in the literature, the comparison of serotonin concentration in strains or with a high fat diet has little published information. The results here present a complex problem. Differences in insulin between the Osborne-Mendel and S 5B/Pl rats offer a possible system for altered LNAA transport and thus a difference in precursor availability and serotonin concentration between strains. Current literature supports this mechanism. There are differences in serotonin concentrations reported with changes in the carbohydrate content of diets. Accumulation of further data will hopefully present a clearer picture.

CONCLUSIONS

These results do not suggest a significant role for steady state serotonin turnover in the response of the Osborne-Mendel and S 5B/P1 rats to a high fat diet. Because serotonin concentrations in both strains responded in a similar manner to the respective diets, these results do not support a relationship between the serotonin concentrations in the strains and the response of each strain to a high fat diet. The differences found in serotonin concentration in a steady state (12 hr fasted) do provide baseline information which may be useful in evaluating acute effects of meal composition on serotonin concentration and turnover in these strains of rat.

FUTURE WORK

Because the original work of Schemmel (Schemmel et al., 1970) was done using a grain base high carbohydrate diet, it would be interesting to have some information on the serotonin concentration and turnover of rats fed a grain based ration.

The response of each strain to a meal of high fat or high carbohydrate composition is another area where further investigation is needed. It is possible that, while each strain shows a similar turnover in a steady state, they could show a different response to a specific meal. The work of Custer (Custer, 1981) investigated this question in relation to a high fat diet, but comparison of a high fat to a high carbohydrate diet has yet to be done.

APPENDIX

Appendix 1

Individual rat body and hypothalamic weights, age, hypothalamic serotonin concentrations per gram tissue and per milligram protein, hypothalamic 5HIAA concentrations per gram tissue and per milligram protein, and ratio of 5HIAA/serotonin.

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
1	OM	CHO	0.439	745	1697	354	151	18.5	2.36	5.8	13.3
1	OM	CHO	0.893	1110	1242	358	151	21.9	2.57	11.1	12.4
1	OM	CHO	0.96	1143	1190	338	151	22.1	2.5	11.4	11.5
1	OM	CHO	1.04	1169	1126	369	151	19.5	2.16	11.7	11.3
1	OM	CHO	1.42	1093	770	370	167	24.5	2.47	10.8	19.7
1	OM	CHO	0.849	583	687	409	167	23.3	2.28	6	17
1	OM	CHO	0.876	559	638	355	167	21.6	2.17	5.6	15.5
1	OM	CHO	1.01	552	546	372	167	21.3	2.23	5.3	12.5
1	OM	CHO	1.39	675	486	349	171	28.5	2.83	6.8	15.1
1	OM	CHO	1.01	640	633	402	171	32.6	3.06	7.3	21.9
2	OM	FAT	0.77	566	736	512	155	24.4	2.72	5.1	6.6
2	OM	FAT	0.909	613	675	454	151	21.2	2.49	5.2	5.7
2	OM	FAT	0.877	545	621	519	151	20.5	2.52	4.4	5
2	OM	FAT	0.642	490	776	448	151	21.3	2.64	4	6.3
2	OM	FAT	0.753	450	598	508	159	27.5	3.06	4	5.4
2	OM	FAT	0.862	710	823	450	159	21	2.89	5.2	6
2	OM	FAT	1.14	757	662	401	159	24	2.96	6.1	5.4
2	OM	FAT	1.3	485	372	486	159	31.1	3.43	4.4	3.4
2	OM	FAT	1.04	631	604	464	159	25.6	3.23	5	4.8
2	OM	FAT	1.4	510	365	495	159	24.7	2.86	4.4	3.2
2	OM	FAT	0.876	523	597	497	159	32.4	3.51	4.9	5.5

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
2	OM	FAT	1.64	715	435	411	167	23.4	2.47	6.8	10.5
2	OM	FAT	1.44	725	503	405	167	27.9	2.67	7.3	14.9
2	OM	FAT	1.45	652	451	517	167	29.4	2.83	6.8	14.2
2	OM	FAT	2.18	785	361	441	167	22.7	2.44	7.3	8.6
2	OM	FAT	3.85	1830	475	364	166	26.6	2.78	17.5	14
2	OM	FAT	5.15	2041	397	492	166	24.4	2.53	19.7	10.3
2	OM	FAT	2	706	354	592	161	27.7	2.45	7.9	9.9
2	OM	FAT	2.39	823	344	472	162	28.1	3.17	7.3	10.2
2	OM	FAT	2.1	1006	478	452	161	21.2	2.87	7.4	10.5
2	OM	FAT	2.07	843	406	412	162	26.1	2.77	8	11.2
2	OM	FAT	1.34	583	434	577	157	26.1	3.13	5.2	12.9
3	S	FAT	0.783	761	972	380	145	32.4	2.05	12	15.4
3	S	FAT	1.69	1398	827	420	145	33.4	2.23	20.9	12.4
3	S	FAT	0.418	642	1536	335	145	27.8	1.68	10.6	25.4
3	S	FAT	1.15	1102	962	310	145	33.3	3.65	10.1	8.8
3	S	FAT	1.28	997	776	370	145	33.1	3.31	10	7.8
3	S	FAT	1.27	1059	834	371	145	45.8	4.82	10.6	8.3
3	S	FAT	2.05	1137	555	363	145	45.3	5	11.4	5.6
3	S	FAT	1.04	822	789	335	145	24.9	2.86	8.2	7.9
3	S	FAT	0.847	918	1084	325	150	22	2.63	9.2	10.8
3	S	FAT	1.01	868	861	370	150	26.2	2.88	8.7	8.6
3	S	FAT	0.916	916	1000	370	150	24.3	2.66	9.2	10

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
3	S	FAT	0.86	851	989	340	150	18.1	2.16	8.5	9.9
3	S	FAT	1.41	689	489	335	158	19.9	-	-	-
3	S	FAT	0.872	563	646	357	158	29.5	3.5	4.8	5.4
3	S	FAT	1.07	500	467	398	158	27.1	3.04	4.5	4.2
3	S	FAT	1.48	622	419	400	158	19.9	2.33	5.3	3.6
3	S	FAT	1.18	472	401	382	158	25.7	3.09	3.9	3.3
3	S	FAT	1.05	561	534	373	158	21.6	2.48	4.9	4.7
3	S	FAT	1.23	540	437	356	158	21.4	2.44	4.7	3.8
3	S	FAT	2.69	869	322	400	166	39.4	3.84	8.9	13.4
3	S	FAT	1.58	524	332	365	166	21.3	1.68	6.6	7.5
3	S	FAT	1.91	674	352	336	166	29.6	2.3	8.7	10.5
4	S	CHO	1.9	1205	634	331	145	20.5	1.51	16.4	8.6
4	S	CHO	0.733	819	1118	310	145	25.3	1.71	12.1	16.5
4	S	CHO	0.762	615	806	302	145	26.2	1.56	10.3	13.6
4	S	CHO	0.67	592	883	295	145	22.6	1.42	9.4	14.1
4	S	CHO	2.49	166	669	327	145	19.6	1.36	23.9	9.6
4	S	CHO	1.08	769	711	330	145	39.3	2.86	10.45	9.7
4	S	CHO	1.35	1050	776	321	145	24.6	1.65	15.6	11.6
4	S	CHO	0.951	821	863	296	145	24.2	1.71	11.6	12.2
4	S	CHO	0.686	1045	1522	391	150	20.2	2.48	10.4	15.2
4	S	CHO	2.41	2173	900	334	150	25.5	2.74	20.2	8.4
4	S	CHO	0.986	1004	1020	202	150	22.9	2.42	9.5	9.6
4	S	CHO	0.815	982	1206	338	150	17	1.96	8.4	10.4
4	S	CHO	1.35	643	478	399	166	23.1	2.7	5.5	11
4	S	CHO	2.69	997	371	341	166	30.9	2.98	10.3	11.9
4	S	CHO	2.3	838	364	367	166	31.6	3.27	8.1	11.1
4	S	CHO	2.71	880	235	313	166	26	3.01	7.6	8.7

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
5	S	FAT	0.972	480	494	253	60	28.3	1.63	8.4	8.6
5	S	FAT	0.718	404	563	174	47	22.7	1.32	7	9.7
5	S	FAT	2.386	952	399	189	47	25.5	1.37	17.7	7.4
5	S	FAT	1.034	452	437	185	47	29.1	1.71	7.7	7.4
5	S	FAT	1.242	518	417	158	47	32.1	1.68	9.9	8
5	S	FAT	1.177	446	379	169	53	24.8	1.77	6.2	5.3
5	S	FAT	1.276	515	403	146	53	28.6	1.97	7.5	5.9
5	S	FAT	1.743	550	316	196	53	21.2	1.53	7.6	4.4
5	S	FAT	1.686	454	269	230	53	20.5	1.52	6.1	3.6
5	S	FAT	1.075	430	400	200	47	25.9	1.95	5.7	5.3
5	S	FAT	1.389	601	436	218	46	16.7	1.39	7.2	5.2
5	S	FAT	1.012	417	412	160	46	26	1.91	5.7	5.6
5	S	FAT	1.071	467	436	189	46	22.7	1.74	6.1	5.7
5	S	FAT	0.764	453	593	174	41	30.9	2.04	6.9	9
5	S	FAT	0.824	415	504	167	41	32.1	1.99	6.7	8.1
5	S	FAT	0.68	424	624	137	41	22.7	1.42	6.8	10
5	S	FAT	0.81	400	494	131	41	26	1.82	5.7	7.1
6	S	CHO	0.839	664	791	155	47	19.4	1.09	11.8	14.1
6	S	CHO	1.27	559	440	161	47	23.5	1.38	9.5	9.5
6	S	CHO	1.184	574	485	162	47	26.6	1.59	9.6	8.1
6	S	CHO	1.008	499	495	162	47	25.4	1.56	8.1	8.1
6	S	CHO	1.132	452	399	198	53	26.4	1.83	6.5	5.8
6	S	CHO	1.372	537	391	192	53	28.7	2.03	7.6	5.5
6	S	CHO	1.2	431	359	203	53	24.5	1.66	6.4	5.3
6	S	CHO	1.659	476	287	205	53	32.7	1.73	9	5.4

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
6	S	CHO	1.598	655	410	196	46	24.7	1.65	9.8	6.1
6	S	CHO	0.973	540	555	193	46	24	1.51	8.6	8.8
6	S	CHO	1.052	448	426	159	46	35.1	2.41	6.5	6.2
6	S	CHO	0.166	454	426	174	46	28.2	2.11	6.1	5.7
6	S	CHO	0.632	469	742	150	41	29.6	2.04	6.8	9.7
6	S	CHO	0.676	517	764	174	41	26.6	1.86	7.4	10.9
6	S	CHO	0.841	478	568	180	41	34.2	1.81	9	10.7
6	S	CHO	1.319	592	449	135	41	46.2	2.96	9.2	7
7	OM	FAT	0.625	430	688	307	67	29.8	1.75	7.3	11.7
7	OM	FAT	0.602	471	782	260	67	33.4	1.86	8.5	14.1
7	OM	FAT	0.667	438	657	302	67	29.9	1.74	7.5	11.3
7	OM	FAT	0.839	523	623	273	67	27.8	1.6	9.1	10.8
7	OM	FAT	0.902	453	502	357	67	33.3	1.92	7.9	8.7
7	OM	FAT	0.942	648	685	303	67	30.4	1.75	11.3	11.9
7	OM	FAT	0.917	527	575	228	66	19.5	1.49	6.9	7.5
7	OM	FAT	0.97	422	435	322	60	23.3	1.64	6	6.2
7	OM	FAT	1.101	459	417	334	60	26.1	1.74	6.9	6.3
7	OM	FAT	0.996	596	598	291	60	24.7	1.72	8.6	8.3
7	OM	FAT	1.167	511	442	277	60	32	2.46	6.7	5.9
7	OM	FAT	1.151	414	360	237	60	20.3	1.69	5	4.3
7	OM	FAT	0.508	330	649	296	60	29.8	2.24	4.4	8.6
7	OM	FAT	1.392	444	319	275	59	31.2	2.26	6.1	4.4
7	OM	FAT	0.499	272	545	241	48	28.8	1.94	4.1	8.1
7	OM	FAT	0.847	379	448	259	48	38	2.54	5.7	6.7
7	OM	FAT	1.045	473	453	232	48	31.9	2.04	7.4	7.1
7	OM	FAT	1.035	461	445	236	48	24.9	1.72	6.7	6.5

TREAT-MENT #	STRAIN	DIET	RATIO	HIAA (ng/g)	HT (ng/g)	LIVEWT (g)	AGE (days)	HYPOTH WT (mg)	HYPOTH PROT (mg)	HIAA/ PRO (ng/mg)	HT/ PRO (ng/mg)
8	OM	CHO	1.035	471	555	179	48	34.9	2.24	7.4	8.6
8	OM	CHO	0.854	481	710	204	48	30	2.05	7	10.4
8	OM	CHO	0.677	392	505	167	48	32.3	2.17	5.8	7.5
8	OM	CHO	1.306	817	626	195	48	32.2	2.16	12.2	9.3
8	OM	CHO	0.827	323	390	238	54	26.2	1.93	4.4	5.3
8	OM	CHO	0.78	408	523	237	60	25.1	1.54	6.7	8.5
8	OM	CHO	0.96	467	486	246	60	23.3	1.91	5.7	5.9
8	OM	CHO	0.489	472	966	267	60	22	1.71	6.1	12.4
8	OM	CHO	1.106	490	443	257	59	28.8	1.99	7.1	6
8	OM	CHO	1.244	508	408	275	60	30.6	2.06	7.6	6.1
8	OM	CHO	0.928	520	560	262	60	27.7	1.85	7.8	8.4
8	OM	CHO	1.212	450	371	248	60	23.8	1.74	6.2	5.1
8	OM	CHO	0.573	389	679	285	60	29.5	1.75	6.6	11.5
8	OM	CHO	0.659	470	713	294	67	23.4	1.14	9.7	14.6
8	OM	CHO	0.801	470	587	281	67	27.4	1.55	8.3	10.4
8	OM	CHO	0.829	619	747	282	67	31.1	1.75	11	13.3
8	OM	CHO	1.04	557	535	300	67	33.5	1.75	10.7	10.2

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