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The Functional Genomics of Weight Loss

For the past 40 years the world has been experiencing and unprecedented increase in size of its westernized citizen. With over 69% of the people in the United States being classified as overweight or obese, unhealthy body weight is undoubtedly an epidemic across the country ("Selected health conditions and risk factors," 2011). Many efforts have been made to reform lifestyle habits and negotiate legislation encouraging healthy practices but to little or no avail. However, there is a relatively new front on which to attack obesity—pharmacology. With microscopic understanding of genes, their proteins, and how to manipulate both, doctors are able to target various physiologic pathways that are pertinent to energy consumption and storage. This paper will compare and contrast two different proteins involved with energy absorption or regulation as well as the respective FDA approved drugs that target them. It will also examine their mechanisms, structures, efficacies, and genetics.

Overview of Obesity Genomics

Involvement of genetic factors in the development of obesity is estimated to be anywhere from 40% to 70%. They may affect body weight by one of two ways: through appetite or through metabolism. There are three ways obesity can manifest itself: monogenic, syndromic, or polygenic. Mono and poly-genic forms are dictated by one or many forms respectively. Syndromic cases manifest with other clinical symptoms. One example is Prader Willi syndrome where the patient experiences peculiar facial features and slight mental retardation. Through animal studies and various genome wide association studies, various genes have arisen as being statistically linked to being overweight or obese.

The first couple genes are involved with leptin pathways. Known to many as the primary satiety signal, leptin is a product of the obesity gene (ob). It regulates food intake through its signaling to the hypothalamus and has blood plasma levels proportional to the amount of adipose tissue is in the body. A nonsense mutation in codon 105 of *Lep* in a mouse model resulted in profound

obesity (X. Yang et al., 2009). A similar case has been observed in humans where the deletion of a guanine nucleotide in codon 133 of *LEP* caused a frame shift mutation, very sparse protein production, hyperphagia, and obesity (Montague et al., 1997). Similarly, mutations in the leptin receptor have caused energy disregulation. Mutations in *LEPR* have resulted in truncated and inactive versions of the leptin receptor. Individuals with this mutation display early onset morbid obesity (Clément et al., 1998). Leptin acts by stimulating POMC neurons.

The POMC gene is located on chromosome 2p23.3 and encodes a propeptide that is posttranslationally modified into adrenocorticotropic hormone (ACTH), MSH (types , , and), and opion receptor ligand - endorphin (Zabel, Naylor, Sakaguchi, Bell, & Shows, 1983). A mutation in the pro-opiomelanocortin gene (POMC) will cause a shortage in all these products. Leptin signals to these POMC neurons which trigger an anorexigenic response (Bell, Walley, & Froguel, 2005). Thus, when this gene is disrupted, so too is its appetite-suppressing effects which predisposes an individual to overeat.

A fourth major gene of interest is the Melanocortin 4 receptor gene (MC4R). It too is member of the G protein-coupled receptor family and signals by activating adenylyl cyclase. This gene has been widely studied as around 100 different mutations have been identified with phenotypic consequences (Kobayashi et al., 2002; Mergen, Mergen, Ozata, Oner, & Oner, 2001). Penetrance of the disease is sometimes incomplete and the clinical expression is variable just as the severity of 100 different mutations.

Pharmaceutical Approach: Two Pathways

Exploring the biochemical mechanisms of weight gain through the pharmaceutical industry offers an interesting perspective. There are two primary routes of targeting energy imbalance and subsequent weight gain. The first is by quelling appetite and the second is by impeding nutrient absorption. The remainder of this paper will explore case examples of each. The first is orlistat, which is trade marked as Alli weight loss pills. The second is Lorcaserin, trade marked as Belviq. The latter drug is unusual in that it targets brain systems with minimal side effects. This is

usually difficult to do as brain systems tend to influence a large number of downstream mechanisms.

Pancreatic Triacylglycerol Lipase

Pancreatic Triacylglycerol lipase (PTL) is a digestive protein responsible for the intestinal absorption by hydrolyzing dietary long chain triglycerides to free fatty acids and monoacylglycerols. It is stimulated by pancreatic colipase as long as it is in the presence of bile salts—a component of bile that is produced by the liver and also aids in the digestion of fats in the small intestine (Lowe, Rosenblum, & Strauss, 1989). The PNLIP gene encodes PTL and is located on chromosome 10q25.3. It is comprised of 13 exons spanning more than 20kb and coding 465 amino acids in the functional protein (Sims, Jennens, & Lowe, 1993). Northern blot analysis reveals that the sole expression of this gene is in the acinar pancreas. People lacking PTL have oily or greasy stools as lipids are not hydrolyzed for absorption.

Experiments and Assays

The first in vitro assay was conducted by Professor Desnuelle to explore the function of PTL. He used a gum Arabic stabilized emulsion of olive oil as a substrate in the presence of bile salts. Porcine pancreatic lipase was purified using protein fractionation by salt and zone electrophoresis. As seen from zone electrophoretic separation of proteins of pancreatic extracts, fractions active against emulsified olive oil showed no hydrolytic activity on solutions of p-nitrophenol esters or tweens. With esters, such as methylbutyrate or triacetin, it was found that purified lipase had no or very little activity on the ester dispersed in water. However, as soon as its concentration was increased above saturation, hydrolysis occurred at a high rate. This suggests that the physical state of the substrate was of prime importance for pancreatic lipase activity (Marchis-Mouren, Sarda, & Desnuelle, 1959).

Interracial activation was confirmed by studying the inhibition of the enzyme by mixed micelles of diethyl-p-nitrophenylphosphate an esterase inhibitor, and bile salt (Maylié, Charles, Gache, & Desnuelle, 1971), and by studying the hydrolysis of water-soluble tripropionin adsorbed onto siliconized glass beads (Brockman, Law, & Kezdy, 1973).

Additionally, pure pancreatic lipase appeared to be inactive on emulsified olive oil in the presence of a micellar concentration of bile salt. Activity could be restored by the addition of a colipase (Borgström, 1973). Colipase was recognized as acting as an anchor for lipase to be adsorbed at bile salt-covered lipid-water interfaces through the specific formation of a 1:1 molar complex with lipase.

There are known regions of the protein that have been proven to be integral to its functionality. When Ser153 or His264 were changed to Leucine, the protein was completely inactivated. Similarly, when Asp177 was substituted with a glutamate or alanine, the protein's activity was reduced by 20% and 80% respectively. It has therefore been deduced that an essential catalytic triad is comprised of Ser153, His264, and Asp177 and is responsible for the hydrolysis of triacylglycerols.

Structure[1]

The structure of pancreatic lipase contains two domains. The N-terminal domain (amino acids 1-336) is a mesh of both - helices and - sheets. The C-terminal domain is mostly - sheets and contains residues 337-449. The enzyme exhibits basket-like form with the sides formed by the hydrophobic residues of each domain to attract nonpolar lipids. At the base of the hydrophobic canyon formed between the two domains is the active site. The ability of pancreatic lipase to hydrolyze ester bonds is due to to three active site residues called "the catalytic triad." The residues of the catalytic triad are Ser153, His264, and Asp177.

A pair of helices lay over the active site, preventing any substrate from entering. This covering, called "the flap," is part of the N-terminal domain, residues 77-86. This covering remains in place until an increase in micelles allows the formation of a lipase/colipase complex. It is an important part of the feedback response that regulates lipid digestion. Until then, the protein will remain unbound to its cofactor and inactive.

Orlistat

1 All structural information from (Lowe et al., 1989)

Orlistat is a weight loss drug that competitively inhibits this protein, will quell the breakdown and absorption of lipids in the small intestine and render these calories unavailable to the body. It is currently marketed as brand names Alli and Xenical. Orlistat is a molecule resembling a naturally occurring lipstatin found in the bacterium *Streptomyces toxytricini* (Barbier & Schneider, 1987). It acts by binding covalently to the serine residue site of pancreatic lipases. At a dosage of 120mg three times a day before meals, Orlistat inhibits lipid absorption by approximately 30% (PDR Network, LLC., 2006).

Pharmacokinetics[2]

Absorption: The systemic exposure of Orlistat is minimal with ^{14}C-Orlistat plasma radioactivity levels reaching their peak 8 hours after oral administration of 360mg. However, blood concentrations of intact Orlistat were only around the limits of detection (<5 ng/mL). In therapeutic studies, the plasma concentrations were sporadic and barely detectable (<10 ng/mL) and showed no signs of accumulation.

Distribution: In vitro Orlistat was >99% bound to plasma proteins such as lipoproteins and albumin. It also minimally partitioned into erythrocytes.

Metabolism: Based on ^{14}C-Orlistat levels, two metabolites make up 42% of the radioactivity in the blood plasma. M1 is the hydrolyzed - lactone ring product of orlistat and M3 is sequential metabolite after M1's cleavage of the N-formyl leucine side-chain. M1 and M3 have significantly reduced lipase inhibitory activity (1,000 and 2,500x less than orlistat respectively). Due to both the reduced activity of the metabolites and the low blood concentration levels, the metabolites are considered pharmacologically inconsequential. M1 has a short half-life of 3 hours while that of M3 is slightly longer at 13.5 hours.

Elimination: After a 360mg dose of ^{14}C-orlistat in both normal weight and obese patients, the major route of excretion was fecal elimination. Major metabolites were also subject to biliary excretion. Approximately 97% of what was administered was excreted in feces and 83% of that

2 All pharmacokinetic information retrieved from (*Xenical-orlistat capsule PPI*, 2009)

was unchanged. Cumulative renal excretion was <2% and complete elimination from the body (both fecal and urinary) took 3-5 days. The half-life of absorbed orlistat ranged from 1-2 hours.

5-HT_{2C} receptor

The 5-HT_{2C} receptor encoded by human gene HTR2C located on the X chromosome[3]. This protein functions most applicably by binding to serotonin and regulates downstream excitatory transmission (Roth, Willins, Kristiansen, & Kroeze, 1998). It is a G coupled receptor for numerous drugs and and psychoactive substances like DOI and LSD. Binding causes a change that induces signaling via G proteins and modulates the activity of downstream affecters. This signaling activates a phosphatidylinositol-calcium second messenger system that modulates the activity of phosphatidylinositol 3-kinase and down-stream signaling cascades. This promotes the release of Ca^{2+} ions from intracellular stores. The displacement of calcium ions activates calcium channels in the brain, releases pro-opiomelacortin neurons and the release of CRH thereby regulating corticosterone levels. Its roles in the regulation of appetite and eating behavior by responding to anxiogenic stimuli and stress will be the mechanism most readily explored for pharmaceutical purposes. The activation of 5-HT_{2C} receptors in the hypothalamus is supposed to activate proopiomelanocortin (POMC) production and consequently promote weight loss through satiety (Cussac et al., 2008; Knauer, Campbell, Chio, & Fitzgerald, 2009).
The 5-HT_{2C} receptor is a multi pass transmembrane protein that is found specifically in the brain tissues.

Lorcaserin

Lorcaserin (trade name Belviq) is a recently approved weight loss drug that targets the central nervous system rather than the more direct absorptive mechanisms like Orlistat. The drug has serotonergic affects and acts like an anorectic by inducing a feeling of satiety. Lorcaserin is a selective 5-HT_{2C} receptor agonist. This receptor plays an important role in modulating monoaminergic transmission, mood, motor behavior, appetite and endocrine secretion, and

3 Polymorphisms in this gene can have different effects on different sexes as a result of its location on a sex chromosome (Stam et al., 1994).

alterations in their functional status have been detected in anxiodepressive states (Millan, 2005). Lorcaserin is remarkably selective for the 5-HT_{2C} receptor. It binds less readily to the other receptors in its class—5-HT_{2A} and 5-HT_{2B} by 18 and 104-fold respectively. This has historically been a very difficult task. Selectivity for the other receptors can cause undesirable side effects such as hallucinations and cardiovalvular insufficiency (Nichols, 2004; Rothman et al., 2000). Scientists observed dose-dependent reductions in food consumption and weight loss in rats. Upon discontinuation of the drug, the rodents' body weights returned to that of the controls. Human 5-HT_{2C} receptors are found mostly in the central nervous system—brain specifically where they control mood cognition and appetite (Roth et al., 1998). Animal studies have found that knock out mice for the 5-HT_{2C} gene experience hyperphagia, increased body weight, leptin resistance, increased adipose deposition, insulin resistance and impaired glucose tolerance (Heisler, Chu, & Tecott, 1998; L H Tecott et al., 1995; Laurence H Tecott & Abdallah, 2003).

Pharmacokinetics

The pharmacokinetics were determined after a dosage of 10mg/kg was administered in male rats. Absorption from the small intestine into the blood plasma was rapid. The mean maximum concentration was 0.760 g/mL after 0.25 hours. Concentrations were around 8-fold higher in the brain than in the blood. The elimination half-life was similar for both plasma and brain levels at around 4.7 hours (Thomsen et al., 2008).

Information as posted on the drug summary indicate that in humans the maximum absorption from the small intestine into the blood stream lies somewhere between 1.5 and 2 hours. Approximately 70% of the administered drug is found bound to plasma proteins. It is assumed that much of the drug is located in the brain where it is difficult to quantify concentrations. The liver breaks down lorcaserin into two metabolites—lorcaserin sulfamate (M1) (major circulating metabolite), and *N*-carbamoyl glucuronide lorcaserin (major metabolite in urine). Finally, the majority of the medication is removed through the renal system (Urine (92.3%), feces (2.2%)) and it has a half-life of 11 hours (Gustafson, King, & Rey, 2013).

Clinical Trials

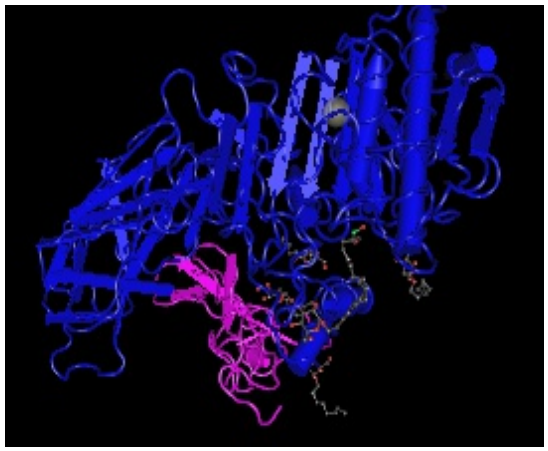
The BLOOM[4] Study Group found very encouraging results. After one year of the trial 47.5% of patients in the test group and only 20.3% in the control group experienced a loss of 5% or more of their body weight (Smith et al., 2010). Another trial replicated these results by finding that 47% of individuals taking a 10mg dosage twice daily lost at least 5% of their body weight compared to the 25% in the control group.

Importance

By identifying genes statistically linked to obesity, scientists are provided with new avenues and new incentives to find biological explanations. The rate of biochemical discovery is therefore accelerated and so too is that of pharmaceutical development. Obesogenic environments found in western cultures are rapidly expanding to other countries. This makes it exceptionally difficult for individuals to stave off one of the most damaging conditions both in terms of personal longevity and quality of life but also of healthcare systems everywhere. It is of utmost importance to quell the appetites or absorptive tendencies of millions, reduce the incidence of life threatening diseases like cancer and heart disease (Calle & Thun, 2004; Eckel, 1997), and improve the quality of life of countless.

Appendix

4 Behavioral Modification and Lorcaserin for Overweight and Obesity Management



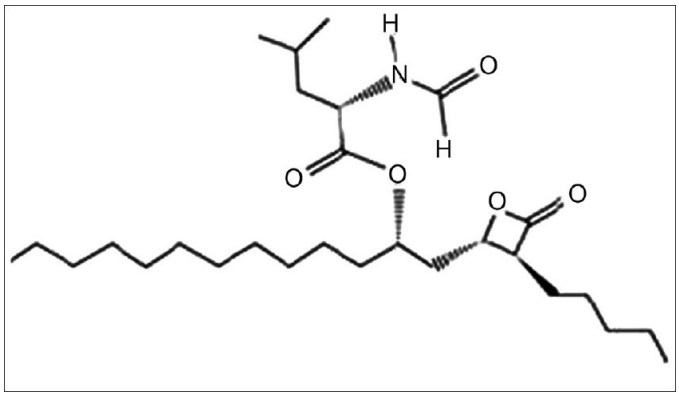


Figure 1: Orlistat structure

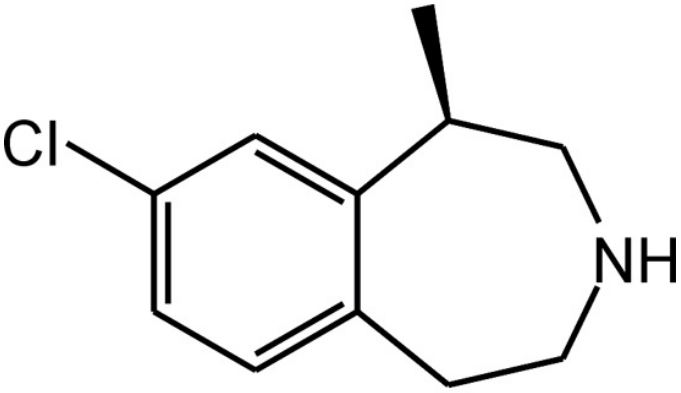


Figure 2: Lorcaserin structure

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