Leptin and its Role in Obesity

Nichole Monhait
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

One of the most taxing issues facing today's society is the obesity epidemic. Humans across the globe are becoming increasingly unhealthy from excess body fat. Obesity differs from simply being overweight in that obesity deals with having too much body fat rather than simply weighing too much17. Prevalence of obesity has more than doubled in the past several decades, and is most notable among American adolescents17. While it is not difficult to locate obese individuals because of their excessive body fat obesity is not only a cosmetic concern. The disease brings health risks such as heart disease, diabetes, and high blood pressure. Individuals who accept their larger bodies aesthetically are then also accepting the countless health risks that come with the disease.

More than one-third of the adult population in the United States was classified as obese in 201017. This number is large, more than doubling obesity rates from thirty years ago17. Immense disparities exist amongst demographics, geographic location, and socioeconomic status. Obesity is most prevalent in Hispanics and blacks and rates are lowest for Asian-Americans17. Extreme obesity is most prevalent amongst women than men, most notably in black women whose rate of extreme obesity doubles that of white and Hispanic women17. Although rates of obesity have plateaued in the past several years, they remain excessively high and the disease continues to be an issue that plagues the population.

The consequences to an individual's health are the gravest part of obesity, but the disease does also have immense costs to society. As obesity rates rise, the economic burden that the disease places on health care system will become increasingly vast. Recently published data suggests that given current trends, 43% of United States adults will be obese by 201818. This translates to a $344 billion increase in disease-related costs18. It is currently estimated that obesity costs nearly $190 billion dollars in healthcare costs, covering 21% of annual spending18.

It is necessary to find suitable therapies and treatments for the disease. Today, treatment primarily revolves around lifestyle changes including dietary, physical activity, and behavioral alterations. Even small changes including healthier eating and exercise can lead to drastic results. While some individuals choose this path and possess the will power required to make those changes, others resort to medications or weight-loss surgery for help.

The causes of obesity are numerous and highly varied. The best known and most talked about also revolve around lifestyle choices. Sedentary individuals are unable to expend the calories they consume, thus accumulating excess body fat17. This becomes an even greater issue when sedentary individuals make poor diet choices. Of course, there are several other contributors to the disease. Heredity plays a role in disease development, although health lifestyle choices are typically sufficient in counteracting its roles. In fact, heredity cannot be solely pinpointed as a cause for obesity17. Rather, inherited genes may predispose an individual to increased weight gain as well as other environmental factors that may contribute to the disease.

Western diet receives a great deal of scrutiny as a major contributor to the increase in obesity rates, specifically in the United States17. Portion control is difficult and vastly skewed in Western diets, which teaches both adults and children poor eating habits. The Western diet is lacking in many essential nutrients such as whole grains, vegetables, fruits, and nuts. Balanced diets help individuals maintain healthy weights. Lack of sleep has also been shown to alter hormones causing individuals to feel hungrier than they may actually be17.

While lifestyle choices do contribute greatly to the disease, a new field of research revolving around individual genetics and hormones that regulate body fat has made great strides in obesity studies. Jeffrey M. Friedman and his lab at The Rockefeller University are in the forefront of this work. Friedman studies the molecular mechanisms that regulate food intake and body weight. His detailed genetic study of mice led to the discovery of leptin, a hormone produced by adipose tissue that plays a key role in body fat regulation16.

Leptin itself is a 16 kDa hormone that acts mainly on the central nervous system9. Leptin receptors are most highly expressed in the hypothalamus but are also located in the brainstem and other brain regions and tissues9. When bound, leptin inhibits feeding and stimulates energy expenditure in order to regulate body weight. The receptors themselves belong to the gp130 family of cytokine receptors, meaning they rely on enzymatic activity of additional proteins in order to initiate signal transduction9.

Leptin acts as a homeostatic regulator in the body. It is secreted in direct proportion to the amount of adipose tissue an individual carries2. An individual with a greater amount of fat will thus secrete more leptin. Under normal conditions, this greater increase in leptin will then cause loss of body fat. These secretions thus moderate food intake, also taking into account energy expenditure. It is then no surprise that defects or malfunction in leptin regulation would then contribute to obesity. One area that receives much attention, particularly from Friedman is leptin resistance by means of elevated plasma leptin levels; hyperleptinemia7. Friedman pursues this avenue because of prior work performed on hormone signaling. As seen in the case of insulin, negative feedback is a typical response to excessive signaling in hormone resistant cases7. He thus proposes that hyperleptinemia paired with high-fat diet is a requirement for eventual leptin resistance, targeting these ideas in a large volume of his work.

There are drastic physiological differences between lean and obese individuals. These differences also translate to mouse models used to study obesity. Although scientists are aware of these differences, the exact events that lead to leptin resistance and subsequent obesity remain up to debate because there may also be other contributors. Those who study obesity often use ob/ob mouse models to replicate leptin resistance7. These mice serve as important research tools because of the many hypotheses surrounding leptin resistance and the development of obesity. Because elevated plasma levels are believed to play a role in the eventual development of leptin resistance, the ability to have models that replicate actual leptin resistance is an important research tool. These mice produce no leptin due to mutation on the ob gene7. Of course, other models have since been implemented. Although the majority of obesity

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cases are linked to increased leptin levels resulting in leptin resistance, there are cases of obesity where leptin levels are diminished and obesity still occurs. For these cases, Friedman was able to cross the widely used ob/ob mouse with a leptin transgene in order to replicate cases where leptin levels are decreased but not entirely eliminated7.

There are two models that may explain how altered leptin sensitivity leads to hyperleptinemia. When uninterrupted, leptin stimulates expression of SOCS-3 protein7. This stimulation has an inhibitory action against leptin signaling, thus downregulation of neuronal SOCS-3 proteins leads to increased leptin sensitivity7. In normally functioning subjects, this regulatory mechanism serves as a protectant against diet-induced obesity. It is then logical to propose that malfunction in this system may directly contribute to cases where obesity is caused by diet alone. In addition to SOCS-3, STAT3 has also been shown to have regulatory impact on leptin signaling. Overexpression of STAT3 has been shown to induce leptin resistance7.

It is also possible that the composition of dietary fats rather than hyperleptinemia are directly involvement in leptin resistance. Friedman proposes that fats may be sufficient to induce leptin resistance by either blocking leptin signaling or initiating a cellular stress process in response to dietary changes7. It is important to note that increased food intake is not the primary target for the development of obesity in these models. In fact, in several of the studies performed, it is not the increased energy intake that leads the development of the disease. Since mice fed high-fat diets do not eat significantly more than those maintained on a normal diet, it is possible that the fats themselves may lead to leptin resistance in some way relating to their composition7. Because of the logic behind both proposals, much of Friedman's work intends to elucidate the exact mechanism behind leptin resistance and subsequent obesity.

It was essential for Friedman to determine a mechanism that would normalize plasma leptin levels. Friedman utilized osmotic infusion pumps in order to clamp plasma leptin to maintain those found in lean wild-type animals. These pumps were able to maintain fixed plasma leptin levels indicative of lean wild-type mice by dispensing leptin at a rate of 150 ng/h which kept leptin levels near 5 ng/mL7.

The resulting study had four groups. The first distinction was plasma leptin levels with ob/ob mice with plasma levels maintained at 5 ng/mL and a control group given PBS in identical pumps7. Next, each of these groups was given either a high-fat diet (HFD) or low-fat diet (LFD). High-fat diets contained 60% calories from fat while low-fat diets had 13% calories from fat7. These diets were maintained for 20 weeks before measurements were taken.

Friedman found no significant difference in food intake between any of the groups placed on the same diet, indicating that valid comparisons could be made and not directly attributed to differences in food intake. Body fat percentages were equally similar. While HFD mice had significantly greater body fat percentage than LFD mice, there was no difference within the two HFD and LFD groups. Thus, obesity results in both HFD groups regardless of mouse model. Importantly, plasma leptin levels differed significantly between groups. Wild-type mice became hyperleptinemic during the course of obesity pathogenesis while ob-normal mice did not7. Although diet-induced obesity followed rather similar progression, the difference in leptin levels was both drastic and notable.

Because obesity follows a rather lengthy time course, it was necessary for Friedman to then detect any long-term consequences of diet on leptin levels. To do so, he first tested how short-term leptin infusion would physiologically alter mice in each of the four groups. Friedman thus replaced his original pumps with ones that either delivered leptin at either the same rate as he started or a rate that would generate a four-fold increase leptin-infusion (450 ng/h higher)7.

As in normal leptin functioning, LFD mice in both groups responded to drastic over-induction of leptin by reducing food intake and therefore losing weight. This did not occur in HFD wild-type mice, indicating that diet-induced obesity may directly cause leptin resistance7. ob-norm mice maintained on HFD remained sensitive to leptin and showed a marked decrease in food intake in response to increased leptin infusion7. Because the only difference between HFD groups was leptin levels, this indicates that hyperleptinemia is, in fact, a requirement for leptin resistance following long-term maintenance on a diet high in fat. Ultimately, Friedman found that a high fat diet is not sufficient to cause leptin resistance. While hyperleptinemia is shown to lead to eventual leptin resistance, it does so by means of downregulating cellular response to leptin7.

Recent attention has also focused on the possible role of leptin receptors in mediating leptin's pleiotropic effects on obesity pathology. To date, six splice variants of the leptin receptor gene have been discovered. While all of these variants are identical extracellularly, they differ at the intracellular C-termini9. The C-terminus, located at the end of peptide chains, varies based on signal transduction ability for leptin specifically9. Thus far, only the ObRb isoform is identified to have signal transduction capability. Because it is the primary functional isoform, mutations to ObRb are hypothesized to play a critical role in leptin signaling and may have important connections to the development of obesity.

In order to determine the consequences of altered ObRa function, Friedman generated an ObRa knock-out mouse model. While ObRb is considered the primary functional isoform, the ability of ObRa to phosphorylate JAK2 and initiate subsequent activation of IRS-1 and ERK indicate the isoform's importance in proper letin functioning9. Creating an ObRa knockout would elucidate consequences from ObRa alone. Friedman then assessed the phenotypic changes that occurred in ObRa knock-out mice compared to wild-type on both a standard and high-fat diet, ultimately finding that changes did occur.

etion of ObRa resulted in significant changes to leptin responsiveness as well as body weight9. While these changes were minor, ObRa knockout mice fed a high-fat diet exhibited a significant decrease in leptin responsiveness and increase in body weight, again indicating the importance of ObRa9. Friedman also found that ObRa knockouts showed a small significant reduction in body weight when exogenous leptin treatment was given. It is then possible that the significant capability of ObRb to alter leptin signaling is dependent on the presence of ObRa and possible all other isoforms. While the role of ObRa may be minor, it may play a role in leptin functioning as demonstrated9. The deletion of ObRb would indisputably cause greater phenotypic consequences, however.

While knowledge regarding leptin signaling and transport is essential to the understanding of its functional role in obesity, Friedman's work is most important because of the potential therapies that may result. In several studies, leptin replacement therapy is implemented as a potential means to reverse obesity pathology. Since obese individuals lose sensitivity to leptin, reintroducing leptin into the body should work to resensitize these individuals, thus allowing leptin to perform its natural homeostatic function7. If this is the case, introduction of leptin should cause obese individuals to increase energy expenditure and reduce caloric intake. Ultimately, leptin replacement therapy should function to reverse the disease.

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In addition to this, Friedman's current works seeks to determine the link between perceived reward value of food and the development of obesity. It is hypothesized that obese individuals allocate reward value of certain foods differently than those who are not and that this does have links to leptin5. In order to determine if this is true, work is being done to uncover the specific mechanisms behind food reward5. It is expected that cravings for high-fat foods is altered in obese individuals which in turn impact body fat composition leading to the development of the disease. There is a demonstrated link between leptin levels and a liking for high-fat foods, however this research is preliminary and required further investigation5.

The development of obesity is linked to many other diseases and risk factors. The amount of fat an individual carries, or adiposity, is also linked to chronic inflammation. It is suspected that this state of inflammation may be a main contributor to the development of other chronic diseases such as heart failure, type 2 diabetes mellitus, or metabolic syndrome10. As already known, malfunction in the homeostatic regulation of body weight occurs potentially from similar inflammatory responses as well as increased cytokine production10. As a result, much of the work that extends the initial findings of Friedman seeks to determine how these diseases and risk factors are actually linked. To date, findings that detect an association between obesity biomarkers and adiposity are minimal.

Fonarow and his team sought to determine the link between obesity, type 2 diabetes mellitus, and metabolic syndrome that often occur in conjunction with heart failure. Specifically, the study aimed to investigate the connection between C-reactive protein (CRP) and leptin in an obese population who already had heart failure, diabetes mellitus, or metabolic syndrome10. To do so, both CRP and leptin were measured in a group of 36 human patients. The findings indicate that a link does exist between CRP, leptin, and body mass index. Patients in the highest body mass index quartile, thus those in the obese range, showed higher CRP levels in comparison to those in the lower quartile10. Ultimately, an association was determined between individuals with heart failure, type 2 diabetes mellitus, or metabolic syndrome, and CRP and leptin10. These findings suggest that CRP may prove to be a suitable prognostic biomarker of obesity as well as other related diseases. Due to the determined link between CRP and leptin and the following inflammatory responses that occur, this research suggests that leptin has further roles that extend beyond involvement in energy balance and hunger control10. Fonarow explains that since leptin receptors are located in regions beyond the hypothalamus and other brain regions, leptin may also play a role in cardiac hypertrophy and elevated leptin levels may also be associated with cardiovascular risks10.

Although most highly expressed in the hypothalamus and other brain regions, leptin receptors are also present in the heart. Because of the distinct link between obesity and an increased risk of cardiac hypertrophy and heart failure, Schafer sought to determine the role of cardiac leptin signaling in obesity. To do so, high-fat diet induced wild-type mice were compared to obese leptin receptor-deficient wild-type mice. In both high-fat diet and genetically modified obese mice, obesity was paired with hyperleptinemia and increased cardiac leptin expression. In wild-type obese mice, there was increased phosphorylation of both leptin receptors and STAT3 in the heart. There was also distinct cardiac hypertrophy in all obese mice8.

To date, the understanding of leptin signaling and its role in obesity has allowed scientists to create a biological link between the disease and its pathology. There is still much work to be done and many new directions that this work needs to go in order to create a full picture. Much of the recent work with leptin extends directly from the work Friedman has done thus far as well as the work that subsequent scientists have performed based off Friedman's discovery of leptin. While leptin signaling, trafficking, and receptor activity are often studied, there is little research regarding the other receptor isoforms and the impact that they may have on this system. In addition to this, little has been done linking leptin signaling to diseases such as heart failure and metabolic syndrome while much has been done that links obesity and diabetes3. In order to properly understand, experiments that looks at these two ideas are both necessary and important. As seen in science many times over, often the smallest players have important significance in transduction pathways and proper signaling. When they are not present, these pathways alter and result in diseases, which could be happening in the case of obesity.

To determine any peripheral importance that may be present from the other splice variants of the ObR receptors would allow scientists to verify that ObRb is, in fact, the most important. Thus far, it is known that ObRb is the most significant in functionality and that ObRa does play a small role in regulating leptin signaling and transport. Because of this, these ideas can be extended to the other receptors. It is possible that the other isoforms also play small but significant roles in normal leptin functioning. To test this, it would be necessary to create knockouts mice for the three remaining isoforms- ObRc, ObR, and ObRe9. These knockouts are critical to the study in order to determine any changes that result in the absence of specific leptin receptors. Each of these knockout strains would then be compared to a wild-type mouse in a variety of different manners. Just as was done for the ObRa knockouts studied by Friedman, these studies would test for changes in leptin responsiveness and body weight while fed a high-fat diet.

In this study, it would be necessary to first quantify total receptor expression in each of the groups to show that the knockouts were successful. Presence of a receptor where there should not be one in a knockout would indicate improper generation of the mouse strain. Following this verification, leptin would be assayed by injecting 8-12 KBq of I-leptin and sacrificing animals several minutes later9. Leptin levels would then be quantified from mouse tissues in order to determine biodistribution. This would be done using RT-qPCR9. Next, a number of different metabolic biomarkers would be tested in order to determine how each knockout altered the mice. These biomarkers would include body weight, food intake, fat versus lean mass, and leptin. These biomarkers would be tested for in both high and low-fat diet knockout mice compared to wild-type controls on the same diet.

It is possible that the peripheral effects here might have something to do with leptin replacement therapy. The current understanding of leptin-mediated obesity is that lack of regulation of leptin homeostasis results in altered leptin secretion from adipose tissue which acts on the hypothalamus, ultimately leading individuals to become overweight. Homeostatic regulation of body weight undoubtedly plays a critical role in the development of the disease. It is predicted that obese individuals lack regulation of leptin or may even be leptin deficient. Recent attention has been placed on potential therapies emphasizing the importance of restored leptin homeostasis through leptin replacement therapy. If correct, this work will significantly impact the understanding of both obesity as a disease but also direct attention to realistic therapies for those who are obese and the necessary targets for these therapies. In order to study this, the previous study will be extended in order to determine how leptin therapy is altered in the absence of certain leptin receptors.

A combination of research tools will be utilized to test this. Specifically, leptin quantification will be performed by ELISA analysis as well as immunohistochemistry. Transgenic scientific methods will be implemented in order to model leptin resistance in an ob/ob mouse model. Due to the implications in its therapeutic value, leptin replacement therapy would then be tested with each of the groups by injecting 800 ng/h subcutaneous leptin for two weeks9. After these injections, the same metabolic biomarkers would be tested for in order to detect any improvement. It is expected that leptin replacement therapy would reverse some of the negative consequences of obesity by reducing food intake and increasing energy expenditure in mice that became obese. The results from these experiments would elucidate the importance of the lesser known leptin receptors and give potential insight into avenues for therapies and further study.

Since its discovery, scientists have invested a great deal of time and money into leptin research. It is without question that the implications that each study brings give hope to the millions of individuals in the world who struggle with obesity. The discovery of leptin and its role in the disease changes the way scientists look at it, moving obesity from a disease caused by lack of self control to a disease with actual psychobiological processes. Application of this work to human subjects will be beneficial in developing potential therapies. While Friedman continues to work feverishly on the topic, others have also joined and have dedicated their time to leptin study as well. There is more to be done but the possibility that a therapy could be developed for obesity is not far off. Friedman's work is ground-breaking and has opened doors that may not otherwise have existed. He has created an entire field of research where there was not one twenty years ago. The discovery of leptin could prove to be one of the most pivotal and important in the twenty-first century and Friedman deserves continued recognition.

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