Synthetic production of exogenous leptin for the treatment of diabetes mellitus

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Diabetes mellitus, a disease affecting one in ten Americans, occurs when cells become resistant to insulin signals that trigger glucose absorption. This results in excess sugar in the bloodstream. Insulin therapy in type 1 and type 2 diabetes is associated with adverse side effects such as fluctuating blood glucose levels, characteristic fat deposits, and increased cholesterol levels. The hormone leptin regulates appetite and blood glucose levels. When administered alone or with insulin to non-obese diabetic mice, leptin minimizes the side effects of insulin therapy or replaces insulin therapy altogether. Our project design includes taking a synthesized or PCR amplified gene for human leptin (LEP) cloned into a plasmid vector and introducing it into *Escherichia coli* using restriction enzymes and DNA ligase. The plasmid will have a strong, inducible promoter and antibiotic resistance for ampicillin. The bacteria will be cultured, and colonies that are resistant to the antibiotic will be further grown. The final culture will receive a chemical signal that instructs that bacteria to make leptin. The leptin is designed with a histidine tag (His-tag) that will facilitate purification using low pressure chromatography. Leptin will be purified to be used as an injectable hormone therapy for non-obese diabetics. Insulin therapy can cost diabetics thousands of dollars each year. By synthetically producing leptin to be used as an injectable therapy, we hope to lower the cost of diabetes treatment and reverse the side effects of prolonged insulin therapy.

Keywords: Leptin, diabetes, insulin, treatment, *Escherichia coli*

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Watch a video introduction by the authors at https://youtu.be/6a0Ynx0V7_Y

Background

Insulin is a hormone found in the pancreas. It regulates blood glucose levels and stores glucose within the liver, muscles, and fat. Because of its role in regulating glucose levels, insulin therapy is currently being used to treat diabetes (Higuera, 2019).

As a consequence of autoimmune malfunction, the body can develop type 1 or type 2 diabetes. Type 1 diabetes is an autoimmune disease that occurs when all insulin-producing cells are destroyed, which results in a complete lack of insulin in the body. Type 2 diabetes is caused by an excessive intake of insulin that results in insulin resistance (Centers for Disease Control and Prevention, 2020). During type 2 diabetes, the body requires more insulin to achieve the same benefits (Higuera, 2019).

Despite diabetes being one of the most common diseases, treatments remain expensive and often lead to adverse side effects. Existing insulin therapy is used to control blood sugar levels in both type 1 and type 2 diabetics; however, it is often associated with side effects of hypoglycemia, excessive weight gain, and mortality (Diabetes, n.d.). Other treatments such as oral medications aim to lower blood sugar levels, although they may sometimes cause side effects of nausea, vomiting, and diarrhea (University of Illinois, 2020).

Many of these adverse side effects, particularly those resulting from insulin therapy, can be reversed through the use of leptin therapy. Leptin is a hormone found in adipose tissue. Its primary function is associated with regulating hunger levels by sending signals to the brain's hypothalamus region (What Is Leptin?, n.d.). However, it also regulates glucose homeostasis and decreases glycemia, insulinemia, and insulin resistance (D'souza et al., 2017). Therefore, injectable leptin therapy has the potential to treat diabetics. Leptin has already been used as a viable treatment for diseases such as dyslipidemia, and has been approved by the FDA (Food and Drug Administration, n.d.). It should be noted that some people can develop leptin resistance, which occurs when the body no longer recognizes signals sent to leptin receptors in the brain. This is often found in people diagnosed with obesity who already have high amounts of leptin circulating in their bodies (Gruzdeva et al., 2019). Therefore, leptin therapy can only be used to treat non-obese diabetics. Our project aims to use leptin as an adjunct to, or a replacement for, insulin therapy (Paz-Filho et al., 2012), because leptin poses fewer adverse side effects when treating diabetes mellitus.

Systems level

Our goal is to transform *Escherichia coli* DH5α to produce leptin. The plasmid will consist of a T7 inducible promoter (BBa_R0184), ribosome binding site (BBa_J611000), leptin producing gene (LEP) with 18 histidine codons (BBa_K2719008), a double terminator (BBa_B0015), and chloramphenicol resistance (See Figures 1 and 4) (Leptin Coding Device, 2018). Transformation using the electroporation technique will transfer the LEP gene into the *E. coli* DH5α cells via the plasmid (Figure 2). The sample will be plated on Luria agar with the chloramphenicol antibiotic and IPTG inducer to isolate a single colony for inoculation. The plasmid adds chloramphenicol resistance to the *E. coli*. Therefore, the only colonies that will grow on the agar are those that are resistant to the antibiotic, and choosing a single colony on the plate will ensure that the colony has a lower chance of contamination or mutations. A micropipette tip will be used to scoop the individual colony from the plate, which will then be added to a flask containing Luria broth. Placing the flask in an incubated shaker overnight will produce the culture ready for protein expression. The overnight culture of the isolated colony will be grown at 37 °C. Adding 0.40 mM of IPTG will then induce transcription of the LEP gene (New England Biolabs, n.d.) by activating the T7 promoter. The leptin produced by the bacteria will contain a polyhistidine tag.

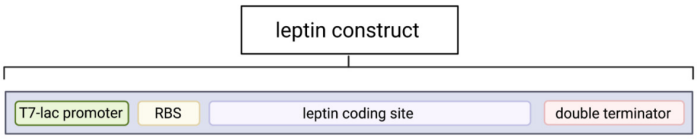

Figure 1. *The leptin construct indicated in the plasmid map consists of the following: The T7-lac promoter, the ribosomal binding site, the leptin coding site, and a double terminator.*

Finally, the induced culture will go through low pressure liquid chromatography using resin-bound Ni2+, which binds the His-tag, and imidazole, which competes with the binding of the His-tag to the immobilized nickel ions, releasing the purified leptin (Figure 3) (His-Tag Purification,, n.d.). This process is the final step in

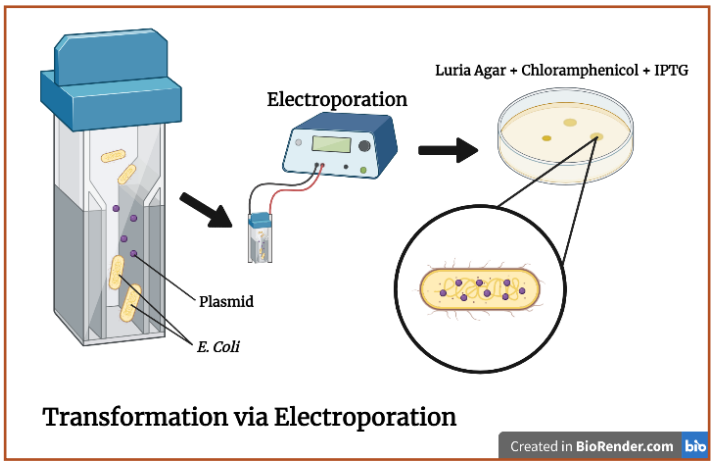

Figure 2. *Through electroporation, the plasmid pLys-M1 (which contains the LEP gene) will be inserted into the E. coli strain DH5α. Following this process, the sample will be plated on Luria agar, containing chloramphenicol and an IPTG inducer, to isolate a colony for protein expression then purification by low pressure chromatography*

preparing and purifying the leptin for further testing to ensure safety for human use.

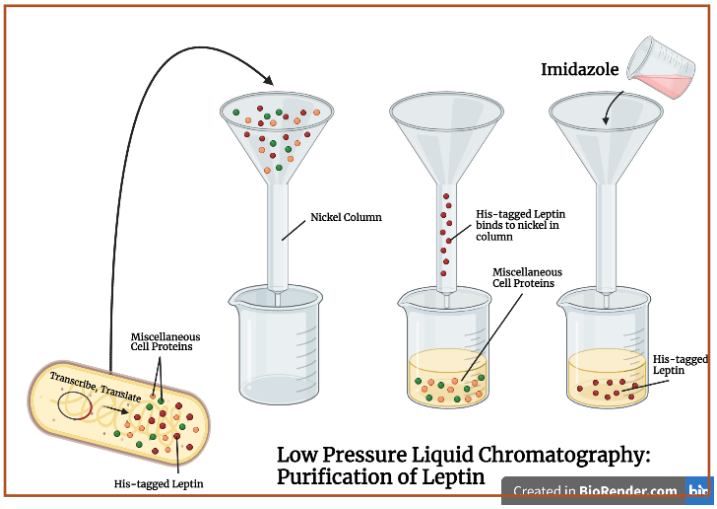

Figure 3. *Low pressure liquid chromatography will be used to isolate the histidine-tagged leptin from the DH5α E. coli strain containing the plasmid. Transformed E. coli from the overnight culture will be lysed using the sonication method, and the lysate (miscellaneous cell proteins, including histidine-tagged leptin), will be loaded onto a nickel column. His-tagged leptin will bind to the nickel ions while other cell proteins will pass through. The leptin will then be eluted, with a high concentration of imidazole to release the purified protein.*

Device level

For our chassis, we chose DH5α *E. coli*. In order to ensure the success of our desired outcome––leptin production––the product must be affordable, easily accessible, versatile, transformation efficient (DH5α Competent Cells, n.d.), and biosafety level 1 (*Escherichia coli* DH5 Alpha, n.d). In the case of mass leptin production, a safe and well-tested chassis would make the best host for the chosen genes. Furthermore, because this is considered a well-tested, lab-safe strain of *E. coli* made for transformation efficiency, it would provide the best accessibility and affordability for mass leptin production.

Parts level

Our goal is to insert the leptin-producing gene (LEP; BBa_K2719008) into the plasmid pLys-M1. We selected this plasmid because it is simple and has many restriction enzyme sites. The plasmid contains a cat promoter and a CmR coding sequence for chloramphenicol acetyltransferase, which confers resistance to this antibiotic. The leptin coding device we selected for insertion into pLys-M1 contains the LEP gene along with a T7 inducible promoter (BBa_R0184), a ribosomal binding site (BBa_J611000), and a double terminator (BBa_B0015; Figure 1) (Sequence Analyzer, n.d.). Once induced, the plasmid will transcribe the LEP gene (Bacterial Transformation, n.d.). Since pLys-M1 is a medium copy number plasmid, we are able to ensure that leptin will be produced without toxicity to the *E. coli* cells due to overproduction of leptin (O'Hanlon Cohrt, 2015). The combination of these characteristics, after the LEP gene is transferred into *E. coli* DH5α through the plasmid, allows the organism to begin producing leptin.

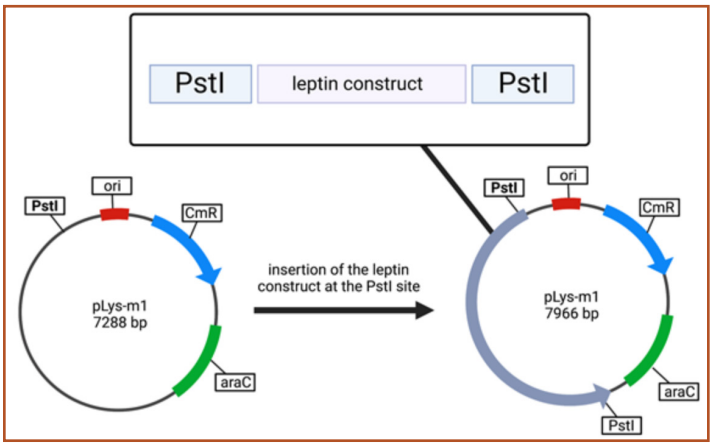

Figure 4. *Using the PstI restriction sites, the leptin construct will be inserted into the pLys-M1 plasmid. The plasmid contains two other important features: the chloramphenicol resistance gene (CmR) and AraC, which encodes for a regulatory protein that controls the metabolic response of the E. coli to arabinose.*

The bacteria will act as a factory to make leptin, which will next be extracted and purified. Upon purification of the leptin, safety tests could be performed to establish its suitability for use in treatment of diabetes mellitus. Attaining safety for the treatment will be easier because the chosen *E. coli* strain is safe and well-tested.

Safety

Within the experiment, the non-pathogenic *E. coli* strain DH5α will be used. This strain is classified as biosafety level 1 (*Escherichia coli* DH5 Alpha, n.d), so it is safe for humans to handle. While handling, individuals will wear gloves and goggles to ensure there is no contamination of the bacterial culture, and to protect themselves. As leptin is naturally produced by adipose cells (fat cells) in the body, it presents no inherent threat to the body. However, too much of the hormone within the human body can lead to leptin resistance, which in turn promotes large amounts of weight gain (Myers et al., 2010). For that reason, proper dosages should be used when administering leptin. For type 1 diabetics, the dosage would be lower as we hope to pair leptin therapy with insulin therapy. In type 2 diabetics, the dosage would be higher, as we aim to replace insulin therapy altogether. This is due to the fact that type 2 diabetics are capable of producing insulin: Therefore, the addition of leptin would work along with existing insulin (The Difference, n.d.). The

appropriate safe and effective dose of injectable leptin is still being evaluated. Multiple medications using the hormone for various purposes, such as weight management and dyslipidemia management, have been approved by the FDA (Food and Drug Administration, n.d.). We acknowledge that many rounds of testing would have to be completed for this to be established as a safe treatment for humans.

Discussions

If testing for this product yields the desired results and gains approval as a medication suitable to treat diabetes, then other options could be placed on the market to make the drug more accessible. Our original project looked into using probiotic bacteria such as Lactobacillus as a carrier for leptin and using this as an oral medication to treat obesity. We ultimately did not pursue this project because many people who are clinically determined as obese develop leptin resistance. We could perhaps develop a product for diabetics using the concept of creating an oral medication to deliver leptin.

However, it should be noted that while leptin is a hormone created in the human body, significant amounts of research would need to be done before it hits the consumer market. People tend to have issues with the concept of injections without long term testing. As a result, we would need to consult expert advisors and health professionals in ensuring the safety of injecting a gut hormone into the human bloodstream, when conducting clinical trials.

As time advances, people could focus on expanding leptin therapy to other health issues as an alternative to typical treatments. For example, leptin has also been identified as a possible solution for weight loss through dietary supplements (Gruzdeva et al., 2019). Furthermore, as research continues, additional naturally-produced hormones besides leptin could be used to manage other diseases as well. The exploration of the previously discussed processes to produce other hormones for hormonal therapy could help future scientists continue the development of similar concepts for treating other diseases.

Next steps

Based on this project's completed research, the next step would involve using electroporation to transform the chosen *E. coli* strain with our plasmid design. After the transformation, processes would be used to test for transformation success and the amount of leptin being produced. Primarily, after designing primers, PCR can be used to detect our gene within the bacteria. Afterwards, processes such as Western blot––utilizing purchased leptin antibodies––would be performed to test if the bacteria are producing leptin.

In the case that leptin production is successful in the transformed *E. coli*, we would proceed with low pressure liquid chromatography to purify the hormone in preparation for future testing.

Author contributions

E.F. and V.M. generated the original idea, while L.A., A.B., K.C., N.H., K.L., and Z.W. helped to fine tune the idea. All authors helped research the background information needed for the design – it was a true collaborative effort. L.A., A.B., E.B., K.C., E.F., K.L., V.M., A.N., C.X., M.Z., and Y.Z wrote and proofread the paper. The key image designers were E.F., C.X., and M.Z., with contributions from all authors.

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