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Oral Administration of Leptin for the Control of Food Intake and Body Weight: Efficiency of the Approach

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Abstract

Upon demonstrating that leptin dissolved in an suitable vehicle and given orally to small rodents and larger mammals reaches rapidly blood circulation and acts on its targets hypothalamic and brown adipose tissues to reduce food intake and control body weight, we evaluated our protocol to enhance the efficiency of the treatment. We found that the presence of anti-proteases and bile salts in the vehicle is imperative. The pH of the buffer should be kept on the basic side in order to counteract proteases in the gastric juice. Addition of amino acids to the vehicle maintains circulating levels of leptin high for long periods of time; this may not beneficial and was thus discarded. Indeed, it may prevent food intake for too long hours and could trigger leptin resistance. Among the several intestinal segments, the ileum was found to internalize leptin more efficiently. Finally, comparing oral administration of leptin with intra-peritoneal or subcutaneous injections has demonstrated that the oral path displays higher efficiencies. This may be due to the fact that while crossing the intestinal cells, oral leptin acquires its chaperon forming a complex with its soluble receptor. The leptin-leptin receptor complex constitutes the normal circulating hormone. Thus oral leptin administered in an optimal vehicle presents great potential for the control of food intake and the maintenance of optimal body weight.

Key words: Oral leptin; Gastric leptin; Efficiency of oral administration

Introduction

Our studies on leptin secretion have demonstrated that this hormone is in fact secreted by several organs; the main ones being the white adipose tissue and the gastric mucosa [1]. By immunocytochemistry we identified the gastric chief cells as being the main source of gastric leptin [2]. Major differences in leptin secretion patterns distinguish the gastric mucosa from the adipose tissue. Indeed, while white adipocytes proceed through a constitutive endocrine secretion of leptin [1,3], leptin release by the gastric mucosa occurs in an exocrine fashion through regulated secretion [1,2]. This implies that white adipocytes discharge leptin continuously without the need of a trigger, while gastric leptin is transiently released only upon stimulation and is secreted into the gastric cavity joining the gastric juice [1]. We have shown in tissue culture that secretion of leptin by adipocytes can be increased but it only becomes significant an hour after continuous stimulation [1,3]. On the other hand, the release of gastric leptin is immediate upon stimulation [1]. Differences in secretory patterns between the two sources of leptin are quite important and related to its functional activities. Leptin secreted by the adipose tissue is vehiculated by the blood stream, crosses the blood-brain barrier by means of saturable transporters [4,5] and reaches its main target hypothalamic cells particularly in the arcuate nucleus. This nucleus contains two populations of neurons expressing leptin receptors with an overall effect on increasing energy expenditure, on activation of the sympathetic nervous system and on the feeling of satiety [6-8]. On the other hand, significant but short lasting release of leptin by the gastric mucosa occurs upon food intake. From the gastric juice, leptin is channeled to the duodenal lumen and crosses the intestinal barrier to reach blood circulation [9]. Upon reaching its hypothalamic target cells it triggers the feeling of satiety thus regulating on a short-term food intake [1,9]. Furthermore gastric leptin through paracrine action controls nutrients absorption and enterocyte metabolism [1]. It activates several intracellular pathways in intestinal cells. It acts on gastric and intestinal epithelial cells potentiating the effect of cholecystokinin, slowing gastric emptying and promoting gastric distension [10,11]. It stimulates production and release of Glucagon-like peptides 1 and 2 (GLP1 & GLP2) that inhibits gastric emptying [12,13]. As we all well know, the feeling of satiety is of short-lasting and is reactivated as soon as gastric leptin secretion is again stimulated through new food intake. Thus, there are two major sources of leptin: a constitutive one arising from adipose tissue which acts on basal and steady state conditions and a gastric one which is of a more narrow acting, targeting intestinal absorption of nutrients, feeling of satiety and overall food intake.

Our cell biology studies have demonstrated that leptin from both the adipose tissue and the gastric mucosa, is secreted via the RER-Golgi-Granule secretory pathways of the corresponding cells; one through constitutive while the other through regulated processes [1,2,14]. The fact that gastric exocrine-secreted leptin, a very small peptide, joins the gastric juice raises serious concern. Indeed, we have shown that free leptin does not resist the harsh conditions of the gastric juice and is immediately degraded [1,15]. Thus, in order to allow this exocrine-secreted hormone to reach circulation, nature has provided a protective chaperon that prevents early degradation. This chaperon was found to be the soluble isoform of the leptin receptor [1,15]. This soluble receptor is synthesized by the same gastric chief cells as leptin, follows the same RER-Golgi-granule secretory pathway and binds leptin at the level of the Golgi apparatus and granules prior release [15]. Further to this, we found that leptin secreted by the adipose tissue is also bound to the same protective leptin-soluble receptor [1,14]. In fact, we have demonstrated that all circulating leptin molecules are bound to the soluble isoform of its receptor [1]. The association

with this soluble receptor not only provides protection against early degradation but it also confers a much longer half-life in circulation [1]. We have previously emphasized that the circulating hormone is not free leptin but rather the complex leptin-leptin receptor [1,15,16].

In what concerns the exocrine-secreted gastric leptin, besides surviving the conditions of the gastric juice, it has to reach blood circulation. We have shown that as soon as 5 minutes after the onset of food intake, or CCK stimulation, circulating levels of leptin raise significantly [1,9,16]. This raise cannot be attributed to the adipose tissue that processes leptin through a slow constitutive secretion; it is rather due to the gastric secretion with a very efficient transport across the intestinal wall via an active transcytotic pathway [9]. Indeed, upon binding the leptin membrane-receptor present on the apical brush-border membrane of the enterocytes, gastric leptin is channelled through clathrin-coated vesicles towards the Golgi apparatus. It gets associated to the soluble leptin-receptor to form again the leptin-leptin-receptor complex prior the transfer to the baso-lateral membrane to be released in the interstitial space of the intestinal mucosa [1,9,16]. Reaching blood circulation across the capillary endothelial wall, leptin is transported to its target cells.

The fact that leptin is normally present in the gastric lumen, vehiculated by the gastric juice to the duodenum and transferred to circulation by crossing the intestinal wall, has triggered the proposal that exogenous leptin given orally could also reach blood circulation and act upon hypothalamic cells for the control of food intake. Upon reaching the gastric juice, oral leptin should follow the very efficient physiological pathway from the gastric cavity to blood capillaries. Once in circulation exogenous oral leptin would reach the hypothalamic target cells to trigger feelings of satiety. Such an approach appears as a powerful avenue for the treatment of patients with low levels of leptin, such as adipose tissue diseases (lipodystrophy), with eating disorders and certain conditions of obesity. Injecting leptin to obese patients was previously tested but with disappointing results [17-20]. In those preliminary studies the amount of injected leptin was quite significant with relatively low impact and a high cost efficiency ratio. We believe that the main issue was the fact that they injected free leptin and not the leptin-leptin receptor complex. Free leptin in circulation has a very short half-life with poor action on hypothalamic target cells. Oral administrated leptin bypass this limitation since as we demonstrated in *in-vitro* and *in-vivo* experiments, leptin acquires its complexed form with the soluble leptin receptor while crossing the duodenal epithelial cells prior its release into circulation [1,9,15,16].

We have tested this hypothesis and administered orally free leptin to normal and obese animals [21-23]. The results obtained have been quite spectacular since significant amounts of oral leptin do reach circulation within the first five minutes after administration [21]. This exogenous leptin was able to control food intake and to induce major loss of body weight in mice [21]. The experiments were carried out on normal mice, on leptin-deficient ob/ob obese mice [24,25] and on leptin receptor-deficient db/db obese mice [24,25]. Force-feeding normal mice with recombinant leptin led to an increase in circulating leptin as early as 5 minutes after its oral administration [21]. Levels decreased thereafter reaching basal values by 60 minutes. Upon oral administration of leptin to mice, their food intake decreased significantly. Once given steadily for several days, it was followed by lowering the animal body weight [21]. Effects of oral administrated leptin on ob/ob leptin-deficient obese mice was even more striking with decreases in food intake of about 60% and a loss of body weight of about 1g a day [21]; the effects of oral leptin being proportional to concentrations given [21]. This indicates that oral administrated free leptin joins the gastric juice, is vehiculated towards the intestinal lumen and crosses efficiently the intestinal wall getting complexed to its receptor before reaching blood circulation and its target hypothalamic cells. Interesting results were obtained when oral leptin was administered to leptin receptor-deficient db/db mice [21]. These obese animals normally display high levels of circulating leptin but lack the corresponding membrane-bound leptin receptors [25]. When db/db obese animals were given oral leptin, the exogenous leptin reached blood circulation but was unable to induce changes neither in the amounts of food intake, nor in their daily increase in body weight [21].

These results indicate that oral administered leptin is able to reach the gastric juice, is vehiculated towards the intestinal lumen, crosses the intestinal wall binding the soluble leptin-receptor thus creating the efficient leptin-leptin receptor complex prior being transferred to circulation and reaching the hypothalamic target cells. In normal animals as well as in ob/ob animals this oral leptin triggered its hormonal response regulating food intake [21]. The results also demonstrated that for leptin (the endogenous or the oral one) to trigger a response, the intact leptin-receptor system must be in place on target cell plasma membrane [21].

Further to these studies, we evaluated the effect of long term oral administration of leptin to mice [21]. Daily oral administration of leptin to normal and obese leptin-deficient ob/ob mice for 30 days, led to decreases in amounts of food intake and stabilisation of their body weight. Histo-pathological and electron microscope examination of the gastric and intestinal mucosa as well as liver tissue of the leptin-treated animals demonstrated no changes in the tissues [21]. These results indicate that oral administrated leptin is able to reach its target hypothalamic cells triggering control of food intake and body weight gain, without causing any major tissue alteration. Encouraged by these results, we carried further studies on larger animals, namely dogs [22]. Results have shown that encapsulated leptin with appropriate anti-proteases and other components, is able to decrease food intake by dogs [22]. Correlations between amounts of circulating exogenous oral leptin and decreases in food intake were highly significant [22].

We further carried out a study on brown adipose tissue, known to be a target of leptin [26]. Upon stimulation by leptin, brown adipose tissue plays key roles in thermogenesis [26-31]; it undergoes lipolysis, decreasing fat synthesis that contributes to the rapid reduction in body weight and adiposity [27,28]. This action is mainly carried out through activation of a mitochondrial protein the UCP1 [26,27,29,31]. Brown adipocyte activity was evaluated after oral administration of leptin and found to be stimulated [23]; number of mitochondria in adipocytes increased significantly displaying numerous cristae with a dense matrix. Several mitochondrial proteins including the UCP1, increased many folds and lipid droplets were fragmented in numerous small ones and got degraded by phagolysosomal activity [23].

Taken together these data clearly demonstrate that once given orally, exogenous leptin is able to cross the intestinal wall and reach through circulation its target cells triggering physiological effects. This prompted us to pursue our study. We decided to evaluate and improve the efficiency of our oral leptin approach. The overall efficiency is related to that of the different steps of the treatment. We know that leptin is a small 16kDa peptide of 146 amino acids and as such, the oral administrated peptide is confronted to major problems when in contact with gastric and pancreatic juices. We have demonstrated that free leptin is very rapidly and totally degraded when in the presence of gastric juice at acid pH [1,9]. Appropriate protection should be provided. Further, it is important to facilitate the transfer from the intestinal lumen to circulation. Thus, we herein report evaluation of the efficiency of the different steps along the pathway taken by leptin from oral administration to circulation in order to survive in the gastric juice and to improve its crossing the intestinal barrier to reach target cells.

Material and Methods and Results

Experiments were performed on normal adult C57BL/6J mice weighting about 20g. Where indicated, ob/ob adult obese mice weighing about 40g were also used. Animals were acquired from Jackson Laboratories (Bar Harbor, ME, USA). Experiments were carried out following the Guidelines of the Canadian Committee of Animal Care and according protocols approved by the Deontology Committee of the Animal Facility of the University of Montreal. Except where otherwise stated, 10µg of recombinant leptin (R&D Systems Inc. Minneapolis, MN, USA) dissolved in 0.5ml of vehicle was administered to mice by force-feeding them. Blood was sampled at the tail vein and evaluation of plasma leptin was carried out using the Leptin Elisa kit (R&D Systems Inc. Minneapolis, MN, USA). All along the study statistical analysis were performed with standard error to the mean and Student's t tests.

Efficiency of the vehicle

The first vehicle tested was a buffer solution containing anti-proteases and bile salts. Composition was as follows: sodium bicarbonate 125mM (pH9), one tablet of anti-proteases (Roche Diagnostic Mississauga, ON, Canada) per 10ml of buffer and sodium deoxycholate 30mM (Sigma St-Louis, MO, USA). Addition of anti-proteases was required to reduce leptin degradation by the gastric juice, while sodium deoxycholate enhances intestinal absorption [32-34]. 10µg of leptin dissolved in vehicle were force-fed to normal mice; levels of circulating leptin were evaluated at time 0, 30 min, one hour and at each hour for a total of 5 hours. Results are reported in figure 1. They demonstrate that administration of the vehicle alone does not have any effect on levels of circulating leptin. Dissolving the leptin in PBS (phosphate buffer saline) without anti-proteases or bile salts also results in an absence of effect, levels of circulating leptin remaining at base line. On the other hand, leptin dissolved in sodium bicarbonate buffer with anti-proteases and bile salts, led to significant increases in levels of circulating leptin with a pick at 30 minutes (Figure 1). Return to basal levels was reached after about three hours.

We then evaluated the efficiency of various buffers. 10µg of leptin were dissolved in different buffers containing the anti-proteases mixture and the bile salts. Figure 2A and 2B illustrate the results obtained. Increases in circulating levels of oral leptin are quite similar with most buffers besides the acetate one that led lower values. Similar raise in circulating leptin is obtained when the pH of the bicarbonate buffer varied from pH 5 to pH 11 (Figure 3). At pH 11, levels of circulating leptin remained high for longer periods but reached basal levels by the second hour (Figure 3). Thus we chose to maintain the pH of the buffer at basic values (pH 9) in order to interfere somehow with the enzymes present in the gastric juice.

We followed by testing the mixture of anti-proteases. Removing it, results in an increase of circulating leptin by 30 minutes but to less significant levels than those obtained with the original vehicle (Figure 4A). We then compared the efficiency of the Roche anti-proteases mixture with aprotinin (Trasylol, Bayer, Leverkusen, FRG) and the results indicated that aprotinin protects leptin in a dose-dependent fashion; aprotinin at 100µg /ml confers similar protection as the Roche Diagnostic mixture (Figure 4B).

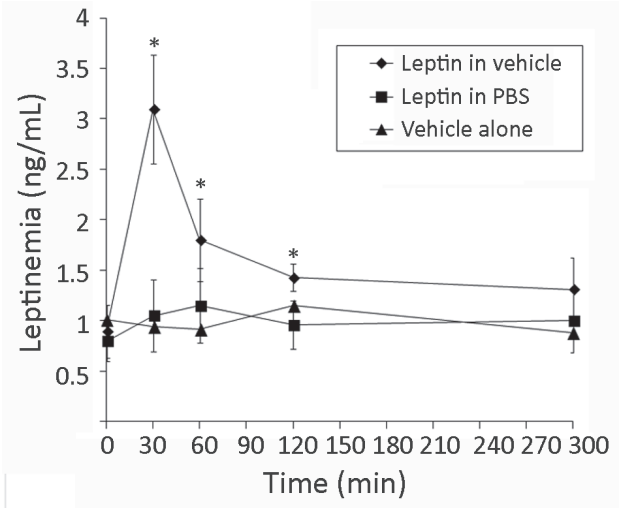

Figure 1: Plasma levels of leptin in C57BL/6J normal mice after oral administration of 10µg of leptin dissolved in 0.5ml of vehicle or PBS. The use of PBS or the vehicle alone does not promote transfer of leptin to circulation. * p<0.05

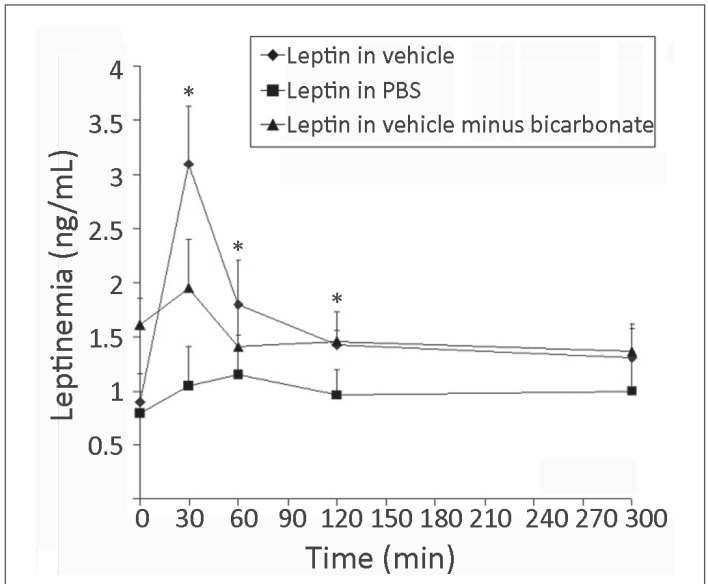

Figure 2: Effect of buffers.
Figure 2A: Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in vehicle, PBS or saline. The use of bicarbonate buffer appears to be significant. * p<0.05

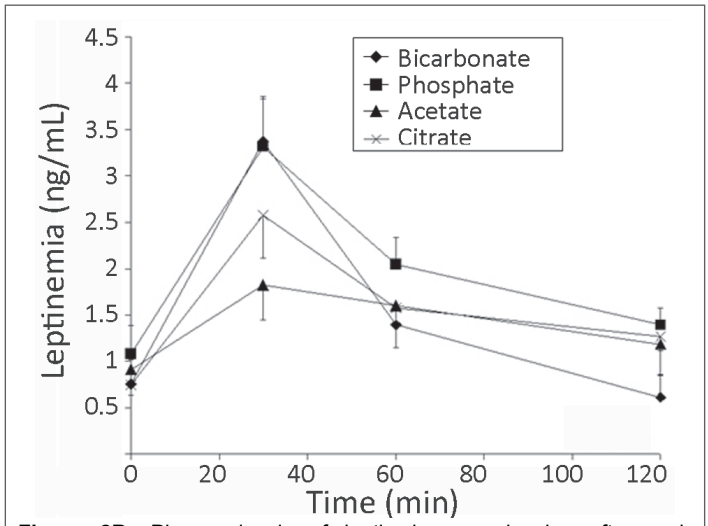

Figure 2B: Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in 0.5ml of vehicle containing anti-proteases and bile salts. Various buffers were tested. Bicarbonate and phosphate buffers yield similar results while acetate buffer appears to be less efficient. * p<0.05

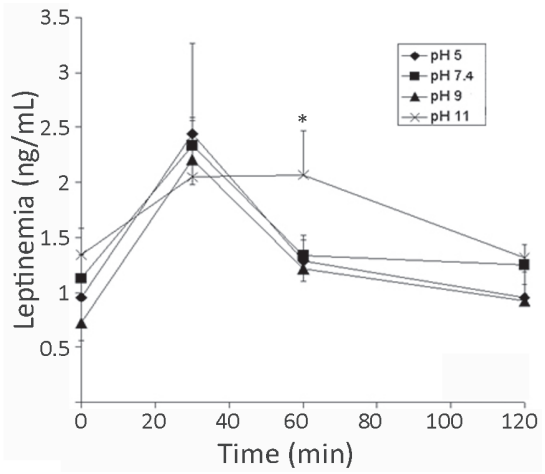

Figure 3: Effect of pH values on leptin absorption. Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in vehicle at different pH values from pH5 to pH11. * p<0.05

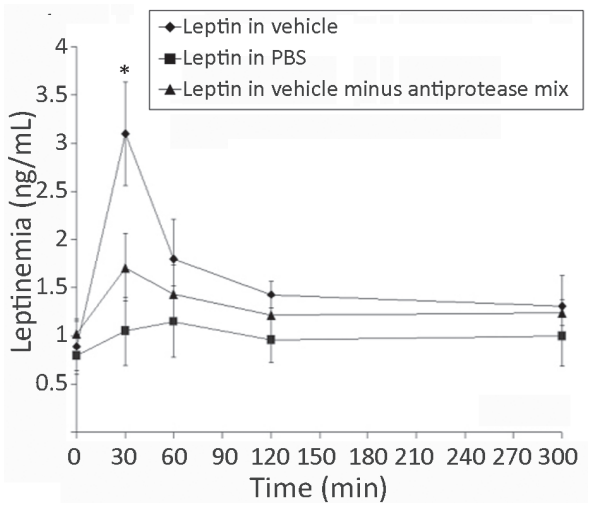

Figure 4: Effect of anti-proteases

Figure 4A: Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in vehicle without anti-proteases. The efficiency of leptin absorption is reduced in absence of anti-proteases. * p<0.05

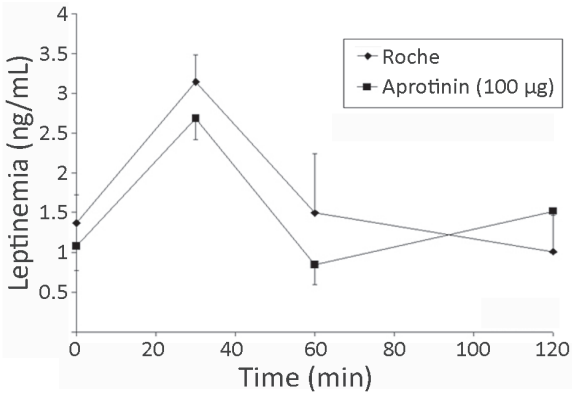

Figure 4B: Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in vehicle containing either the anti-proteases mixture of Roche or 100µg of aprotinin. Both anti-proteases solutions appear to have similar effects.

Finally we tested the efficiency of different bile salts (Figures 5 and 6). Removing the bile salts from our vehicle results in an absence of effect; levels of circulating leptin remained at base line (Figure 5). Comparing deoxycholate with taurocholate, cholate or lithocholate led us to conclude that all yield quite similar results (Figures 6A, 6B, 6C); all the bile salts tested stimulate leptin absorption by the duodenal cells with similar efficiencies.

In our search of an optimal vehicle for oral leptin delivery, we carried out an additional test. We have previously demonstrated that amino acids stimulate secretion of leptin by the adipose tissue [1,35]. Thus, we added to our vehicle 500µM of glutamine. This resulted in significant increases in levels of circulating leptin (Figure 7). The presence of amino acids in the vehicle maintains leptin levels high for long periods of time up to five hours. As discussed later, these high levels could derive from stimulation of leptin secretion by the adipose tissue but might however not be beneficial for the individual.

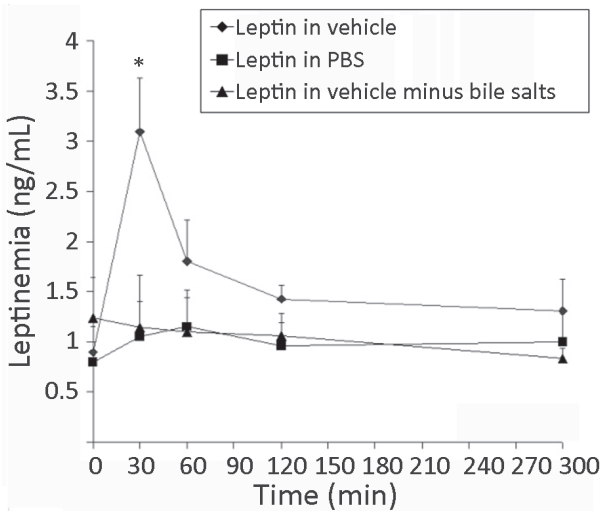

Figure 5: Effect of bile salts: Plasma levels of leptin in normal mice after oral administration of 10µg of leptin. Removing the bile salts resulted in an absence of leptin absorption.* p<0.05

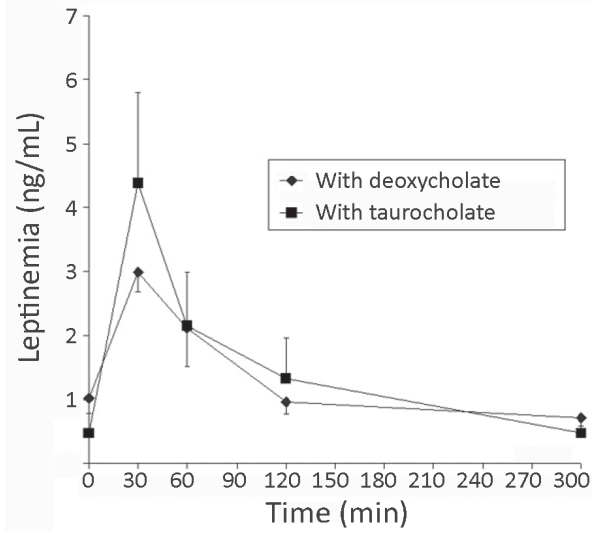

Figure 6: Effect of bile salts

Figure 6A: Replacing the deoxycholate by taurocholate increases absorption of leptin but this was not highly significant

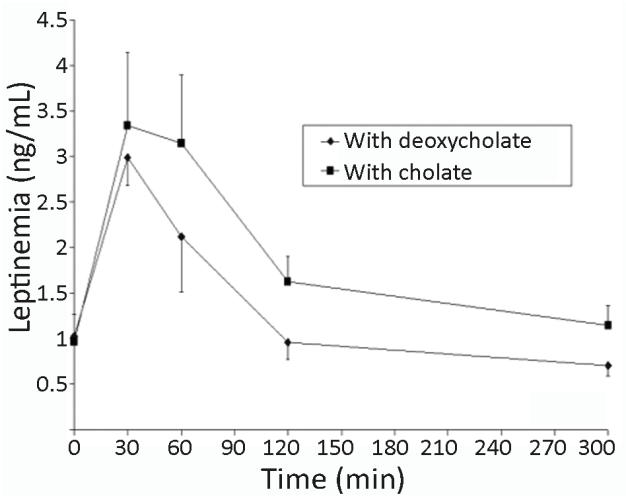

Figure 6B: Replacing deoxycholate by cholate did not lead to significant improvements

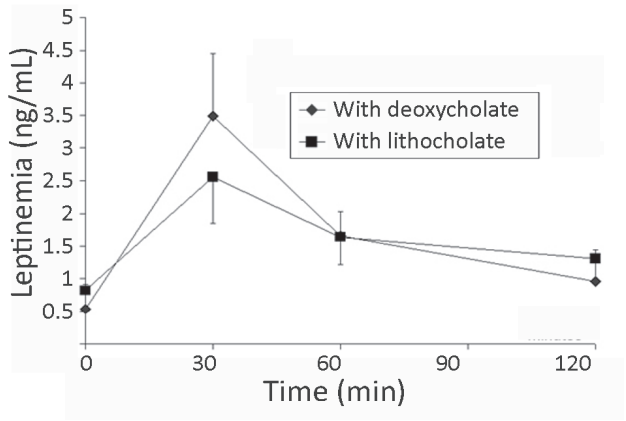

Figure 6C: Replacing deoxycholate by lithocholate did not lead to significant improvements

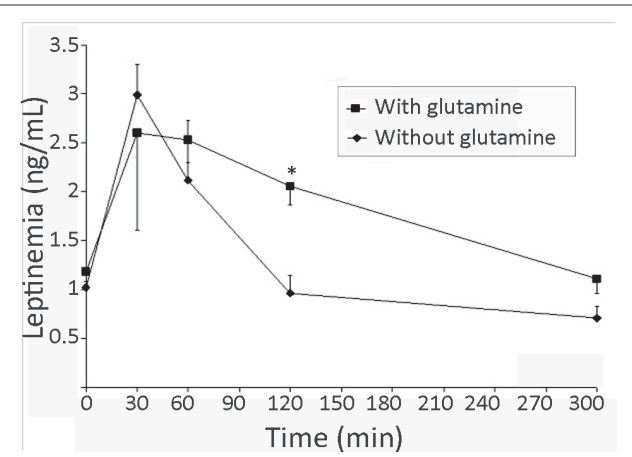

Figure 7: Addition of amino acids. Plasma levels of leptin in normal mice after oral administration of 10µg of leptin in vehicle containing 500µM of glutamine. The presence of the amino acid led to high levels of circulating leptin for longer periods of time.* p<0.05

Efficiency of the intestinal absorption

In the next step we evaluated the segment of the intestine that internalizes and transfers leptin toward blood circulation most efficiently. To carry this experiment leptin was dissolved in its vehicle and inserted in the lumen of different portions of the intestine. Mice were anesthetized and the abdominal cavity exposed. Segments (each 5cm long) of duodenum, jejunum or ileum were selected and isolated in situ from the rest of the digestive tract by placing two clamps while maintaining the digestive track intact. The same volume of vehicle (0.5ml) containing leptin (10µg) was inserted in the lumen of each segment and blood was sampled at regular time points during the first hour. Experiments were carried out on 15 animals (5 for each of the intestinal segment). Results are reported in figure 8. They show that leptin absorption occurs all along the small intestine, the ileum being by far much more efficient than the duodenum or the jejunum. This result is quite significant in the context of designing an eventual «*leptin pill*». However, we should be aware of the many factors that can influence these experiments. Indeed, the ileal loops are located far from the duodenum and might thus contain rather low levels of pancreatic proteases. On the other hand, due to the presence of microvilli at the apical plasma membrane of the intestinal cells, the actual absorbing cell membrane area and differences between duodenal jejunal and ileal surfaces are difficult to evaluate.

Efficiency of methods of administration

Finally, to demonstrate the efficiency of our oral administration approach, we carried out an experiment to compare absorption of leptin through four different paths. We assessed levels of circulating leptin after oral administration versus subcutaneous injection (interscapular), versus intra-peritoneal injection, versus intravenous injection (tail vein).

For the oral administration, 10µg of leptin were dissolved in the optimal vehicle, including anti-proteases and bile salts, and force-fed to normal C57BL/6J mice (5 animals per experiment). For the subcutaneous, intra-peritoneal and intravenous injections, 10µg of leptin dissolved in saline were injected to the animals. Blood was sampled at 5 minutes intervals during the first 30 minutes and then at every 15 minutes for a total of two hours. Results show that absorption of oral leptin is very efficient and occurs quite rapidly (Figure 1). As expected subcutaneous, intra-peritoneal and intravenous injections of leptin led to very high levels of circulating leptin that dropped rapidly (Figures 9A-9C). However, in spite of the high circulating levels, the physiological efficiencies of the different delivered leptins remain to be assessed.

Experiments to assess the overall biological efficiency of circulating leptin were carried out for a period of three days. Various concentrations of leptin were tested. For oral administration 2.5µg and 10µg of leptin dissolved in the optimal vehicle were forced-fed once a day for three days. For intra-peritoneal injection, 2.5µg, 30µg or 120µg of leptin dissolved in saline were injected once a day for three days. For subcutaneous injection only one concentration, 120µg of leptin in saline was given once a day for three days. Repeated intravenous injections in the tail vein proved to be quite difficult painful and problematic; we thus decided not to complete the experiment through this approach. Each experiment was carried out on 5 normal mice. Body weight changes were recorded every day. Results are summarized in figure 10 and demonstrate that there are major differences in the biological efficiency of the delivered leptin. The differences are related to the method of leptin administration. Orally-administered leptin appears to be by far the most efficient. Indeed, at the end of the 3 days experiment, the animals force-fed with 2.5µg of leptin lost 2.5g while those receiving 10µg lost 4g (Figure 10). These changes are more significant that those obtained upon subcutaneous or intra-peritoneal administrations

(Figure 10). The rational of those differences lies on the fact that upon reaching the gastric juice, the oral administered hormone uses the normal physiological in situ path for crossing the intestinal wall and reach blood circulation. This will be considered in details in Discussion.

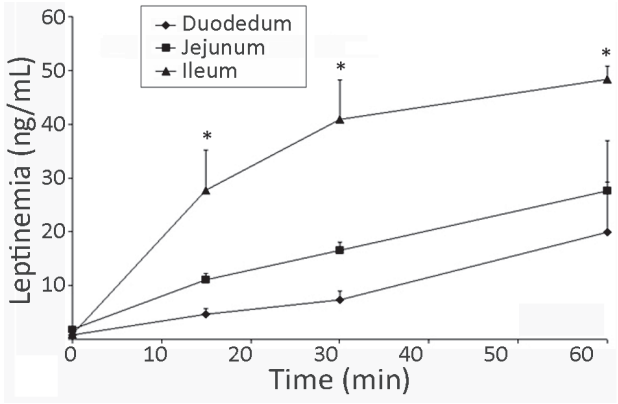

Figure 8: Efficiency of the intestinal segments. 10µg of leptin in 0.5ml of vehicle was inserted in the lumen of the duodenum, jejunum or ileum. Loops 5cm-long were selected for each of the segments. 5 experiments were performed for each of the intestinal segments. Transfer of leptin from the lumen of the intestine to blood circulation differs significantly from one segment to the other. Ileum appears to be the most efficient in transferring leptin from the lumen to circulation. * p<0.05

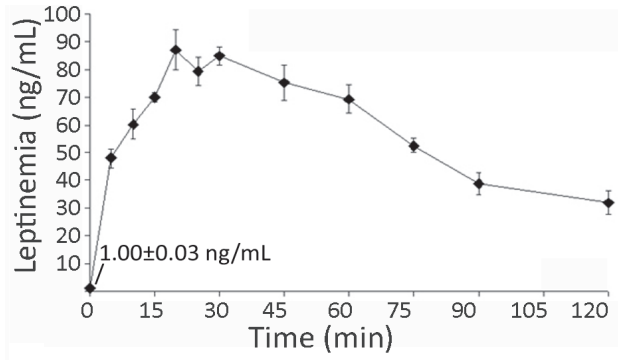

Figure 9: Blood levels of leptin following its injection at different sites.
Figure 9A: Levels of circulating leptin upon subcutaneous (inter-scapular) injection of 10µg of leptin in saline to normal mice (N=5). Blood levels rise rapidly within 5 min

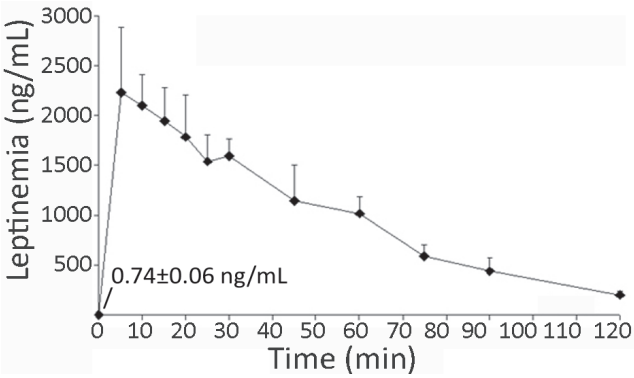

Figure 9B: Levels of circulating leptin upon intravenous (tail vein) injection of 10µg of leptin in saline to normal mice (N=5). Blood levels rise rapidly.

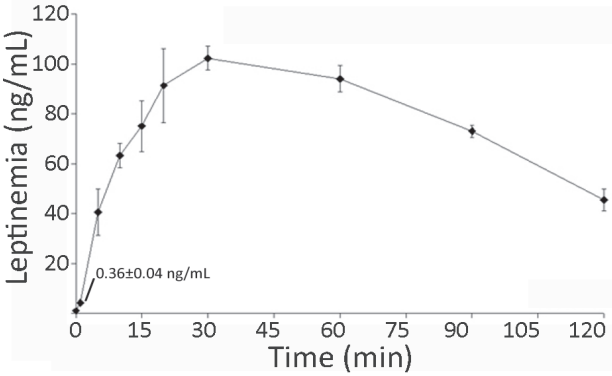

Figure 9C: Levels of circulating leptin upon intra-peritoneal injection of 10µg of leptin in saline to normal mice (N=5). Blood levels rise rapidly

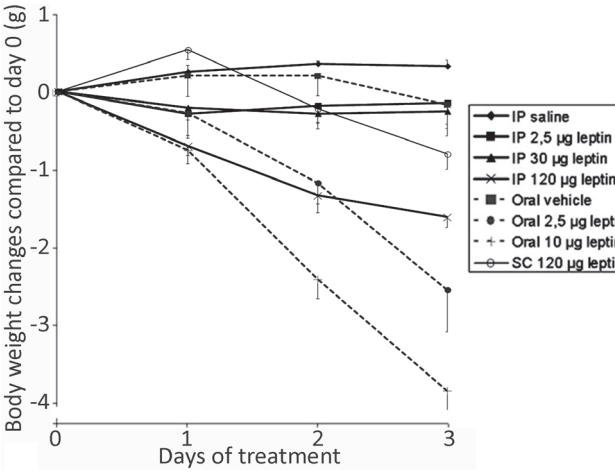

Figure 10: Comparative evaluation of leptin efficiency according to its way of administration. Loss of body weight after three days of daily administration is reported. Oral administration appears to be significantly more efficient in lowering body weight than intra-peritoneal or subcutaneous injections. IP: intra-peritoneal injection; SC: subcutaneous injection.

Discussion

We have previously demonstrated that the gastric mucosa is responsible for the secretion of leptin, a potent regulator of food intake [1,16,36,37]. Indeed, upon starting our meals, the gastric chief cells are stimulated and discharge leptin into the gastric cavity. This leptin is bound to a protecting chaperon which we found to be the soluble isoform of the leptin receptor [15]. Thus, the leptin-leptin receptor complex released by the chief cells joins the gastric juice and is vehiculated to the intestinal lumen. Upon reaching the luminal membrane of the enterocytes, leptin binds to its membrane bound receptor on the apical brush-border membrane of the enterocyte and is internalized. Through the endosomal compartment of the intestinal cells, leptin is channeled to the Golgi apparatus where it binds again to the soluble isoform of its receptor prior being released at the baso-lateral membrane towards blood circulation. The leptin-leptin receptor complex reaches its hypothalamic target cells triggering the feeling of satiety and controlling food intake. Secretion of such an important hormone by the gastric mucosa is confronted to many pitfalls, in particular the harsh conditions of the gastric juice and the transcytotic pathway across the intestinal epithelial cells. Besides protecting the peptide from early degradation, the binding to the leptin soluble receptor confers a

longer half-life to the peptide [15]. This longer-half life allows the peptide to be vehiculated towards the intestinal lumen, to cross the intestinal wall and to join circulation to finally reach its target cells. The binding to the soluble receptor is not unique for the gastric leptin, it also applies to leptin secreted by the adipose tissue [1,16]. We have demonstrated that the circulating hormone is not the free peptide but rather the complex leptin-leptin receptor [1, 16].

The fact that gastric leptin is secreted in an exocrine fashion into the gastric cavity prompted us to put forward the possibility of having an oral administration of leptin that would increase its circulating levels [21]. Indeed, oral leptin might be of great significance and a powerful avenue to regulate food intake in order to decrease body weight in conditions of obesity. Given properly, it will also allow for maintaining body weight at appropriate levels. In previous studies, we have demonstrated that oral administration of leptin is indeed able to reduce food intake, to trigger decreases in body weight and when properly administered to stabilize body weight for long periods of time [21].

In our first report on oral leptin [21], we have shown that its administration to mice induces significant decreases in food intake with losses of body weight. Changes were closely correlated to amounts of oral leptin. Adjusting those amounts enabled the animals to reduce, maintain and stabilize their body weight for long periods of time [21]. Carrying experiments with normal, ob/ob leptin-deficient mice and db/db leptin-receptor-deficient animals have demonstrated that the oral administration of leptin is particularly efficient in ob/ob mice reducing their food intake and maintaining a rather normal body weight for long periods [21]. In contrast the db/db animals did not respond to the oral leptin which implies that the mechanism for oral leptin action requires a fully functional leptin receptor system at the plasma membrane of the target hypothalamic cells [21]. We have also demonstrated that oral administration of leptin for a long period of time did not induce any alteration of the gastric or duodenal mucosa, nor on liver tissue [21]. Further to the studies performed on small rodents, oral leptin was administered to larger mammals with similar results [22]. Upon administration of a leptin pill to dogs, the animals reduced their food intake by 15 to 55%. Variations in the efficiency were related to the time of the day in which the experiments were performed; mornings being much more efficient than afternoons [22]. Finally we demonstrated that brown adipose tissue a target for circulating leptin [26-29] is also stimulated by oral leptin [23]. Brown adipocytes responded to the oral leptin by increasing lipolysis and decreasing fat synthesis leading to reduction of adiposity [23]. Thus oral administration of leptin is proposed and is anticipated as a promising avenue for the management of food intake and for the control and maintenance of optimal body weight.

Success of this oral approach is based on optimisation of its administration. Several pitfalls had to be circumvented in order for oral leptin to reach its targets hypothalamic cells. The vehicle in which the hormone is administered should be able to play key roles in protecting the hormone from early degradation and to stimulate its transfer to blood across the intestinal wall. We report herein some of the assessments performed in order to establish an optimal vehicle.

Several buffer solutions were tested revealing that most common buffers are adequate; bicarbonate and phosphate yielding similar optimal results. pH values of the buffer had little influence provided that they are kept on the basic side to counteract the acidity of the gastric juice needed for gastric enzymes activity. On the other hand, the presence of anti-proteases and bile salts was crucial. We reported previously that in absence of protection, the leptin peptide is rapidly degraded by the gastric juice [1]. The anti-proteases protect the small peptide from early degradation by the gastric and pancreatic juices. For endogenous leptin, this protection is afforded by the binding with the soluble isoform of the leptin receptor. Once in the duodenal lumen the protection persists to prevent degradation by pancreatic proteases. Our tests have demonstrated that either the Roche anti-proteases mixture or just aprotinin are able to provide sufficient protection against enzymatic degradation. Bile salts stimulate internalization by intestinal cells [32-34]. As for the buffer, several bile salts were tested and have shown to confer similar promotion for internalization. Since we reported previously that amino acids are able to stimulate leptin secretion by adipocytes [1,35], an additional test was carried out. Following the rational that amino acids would stimulate leptin secretion by the adipose tissue and promote combined actions of exogenous oral leptin and endogenous adipose tissue-secreted leptin, we added glutamine to our vehicle. This resulted in increasing levels of circulating leptin. However, since adipose tissue is long to respond to stimulation and processes leptin through a constitutive secretion [1,3], levels of circulating leptin remained high for long periods of time; maybe too long periods. The proposition of adding amino acids to the vehicle was thus discarded since our aim is to promote rapid but short lasting effects on food intake. Keeping high levels of circulating leptin for long periods may maintain the feeling of satiety for too long, preventing normal and regular food ingestion. On a long term, it might also trigger leptin resistance which will go against our main objective. The proposal of adding amino acids to our vehicle was not implemented.

Thus, our quite simple vehicle consisting of anti-proteases and bile salts in an appropriate buffer has shown to be quite efficient.

In order to design the optimal capsule or pill for oral administration of leptin, we needed to evaluate the absorption efficiency of the different segments of the intestine. For such an experiment, we introduced same amounts of leptin dissolved in the optimal vehicle into the lumen of the duodenum, jejunum or ileum to find out that the latest displays a higher capacity for transferring leptin to blood circulation. This experiment and the results obtained are difficult to analyze and do not yield definite results since many unknown intrinsic factors could not be avoided. Indeed, due to its structure, the absorbing surface of the intestinal cell membrane is very difficult to assess. In spite of introducing the same amount of leptin into intestinal segments having the same length, the characteristics of the epithelial luminal membranes (microvilli) makes it extremely difficult to measure real absorbing surfaces. Differences in amounts of luminal pancreatic enzymes among intestinal segments also interfere with proper interpretation of the results.

Last but not least, tests were performed to compare pathways of leptin administration. We compared oral administration with subcutaneous and intra-peritoneal injections. While circulating levels of leptin are by far higher upon injection, we found out that leptin given orally is significantly more physiologically active than the injected ones. As clearly mentioned in previous reports [1,16,36], the circulating biological active hormone is not the free leptin peptide but rather the leptin-leptin receptor complex. Thus, by injecting the free peptide, the obtained biological action was weak. In contrast the oral administered leptin needs to cross the intestinal wall and by doing so, gets associated to its soluble receptor. Consequently, the oral leptin circulates in blood and reaches its target hypothalamic cells in its complexed form and is thus more stable, interacting with hypothalamic cells through normal pathways with optimal efficiency.

We can conclude that in terms of leptin administration to patients two avenues are possible:

- Injection of leptin: In this case we believe that injecting the leptin-leptin receptor complex is mandatory; or
- Oral administration of leptin: In this case the leptin pill could consists of either a- the leptin-leptin soluble receptor complex or b- the free leptin combined with anti-proteases and bile salts.

Our results have shown that the later is indeed simpler, straightforward with excellent results.

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References

1. Cammisotto PG, Levy E, Bukowiecki LJ, Bendayan M (2010) Cross-talk between adipose and gastric leptins for the control of food intake and energy metabolism. Prog Histochem Cytochem 45: 143-200.
2. Cammisotto PG, Renaud C, Gingras D, Delvin E, Levy E, et al. (2005) Endocrine and exocrine secretion of leptin by the gastric mucosa. J Histochem Cytochem 53: 851-860.
3. Cammisotto PG, Bukowiecki LJ (2002) Mechanisms of leptin secretion from white adipocytes. Am J Physiol Cell Physiol 283: C244-C250.
4. Kastin AJ, Pan W, Maness LM, Koletsky RJ, Ernsberger P (1999) Decreased transport of leptin across the blood brain barrier in rats lacking the short form of the leptin receptor. Peptides 20: 1449-1453.
5. Koistinen HA, Karonen SL, Livanainen M, Koivisto VA (1998) Circulating leptin has saturable transport into intrathecal space in humans. Eur J Clin Invest 28: 894-897.
6. Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, et al. (1996) Localization of leptin receptor mRNA and of the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett 387: 113-116.
7. Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB (1998) Distribution of leptin receptors mRNA isoforms in the rat brain. J Comp Neurol 395: 535-547.
8. Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404: 661-671.
9. Cammisotto PG, Gingras D, Bendayan M (2007) Transcytosis of gastric leptin through the rat duodenal mucosa. Am J Physiol Gastrointest Liver Physiol 293: G773-G779.
10. Moran TH, Ameglio PJ, Schwartz GJ, McHugh PR (1992) Blockade of type A not type B CCK receptors attenuates satiety actions of exogenous and endogenous CCK. Am J Physiol 262: R46-R50.
11. Wang L, Barachina MD, Martinez V, Wei JY, Tache Y (2000) Synergistic interaction between CCK and leptin to regulate food intake. Regul Pept 92: 79-85.
12. Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, et al. (1997) Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 273: E981-E988.
13. Naveilhan P, Hassani H, Canals JM, Ekstrand AJ, Larefalk A, et al. (1995) Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nat Med 5: 1188-1193.
14. Cammisotto PG, Bukowiecki LJ, Deshaies Y, Bendayan, M (2006) Leptin biosynthetic pathway in white adipocytes. Biochem Cell Biol 84: 207-214.
15. Cammisotto PG, Gingras D, Renaud C, Levy E, Bendayan M (2006) Secretion of soluble leptin receptors by exocrine and endocrine cells of the gastric mucosa. Am J Physiol Gastrointest Liver Physiol 290: G242-G249.
16. Bendayan M, Cammisotto P (2016) Leptin secretion by adipose tissue and gastric mucosa for the control of food intake: A review. Austin J Endocrinol Diabetes 3: 1048.
17. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, et al. (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 282: 1568-1575.
18. Rosenbaum M, Sy M, Pavlovich K, Leibel RL, Hirsch J (2008) Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli J Clin Invest 118: 2583-2591.
19. Trevakis JL, Lei C, Koda JE, Weyer C, Parkes DG, et al. (2010) Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats. Obesity 18: 21-26.
20. Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, et al. (2010) Leptin therapy in insulin-deficient type 1 diabetes. Proc Natl Acad Sci USA 107: 4813-4819.
21. Bendayan M, Cammisotto PG (2016) Control of food intake and body weight through oral administration of leptin. Austin J Endocrinol Diabetes 3: 1050.
22. Bendayan M, Cammisotto PG (2016) Control of food intake by oral administration of leptin to dogs. Austin J. Endocrinol. Diabetes 3: 1050.
23. Bendayan, M, Cammisotto PG (2016) Activation of brown adipose tissue by oral administration of leptin. Austin J Endocrinol Diabetes 3: 1051.
24. Coleman DL, Hummel KP (1967) Studies with the mutation diabetes in the mouse. Diabetologia 3: 238-248.
25. Coleman DL (1978) Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14:141-148.
26. Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, et al. (1997) Direct effects of leptin on brown and white adipose tissue. J Clin Invest 100: 2858-2864.
27. Fedorenko A, Lishko PV, Kirichok Y (2012) Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. Cell 151: 400-413.
28. Yi CX, Meyer CW, Jastroch M (2013) Leptin action in brain. How and when it makes fat burn. Mol Metab 2: 63-64.
29. Commins SP, Watson PM, Frampton IC, Gettys TW (2001) Leptin selectively reduces white adipose tissue in mice via a UCP1-dependant mechanism in brown adipose tissue. Am J Physiol Endocr Metab 280: E372-E377.
30. Kortelainen ML, Pelletier G, Ricquier D, Bukowiecki LJ (1993) Immunohistochemical detection of human brown adipose tissue uncoupling protein in an autopsy series. J Histochem Cytochem 41: 759-764.
31. Kozak LP, Anunciado-Koza R (2008) UCP1: its involvement and utility in obesity. Int J Obes (Lond) 32 Suppl 7:S32-S38.
32. Bendayan M, Ziv E, Gingras D, Ben-Sasson R, Bar-On H, et al. (1994) Biochemical and morpho-cytochemical evidence for the intestinal absorption of insulin in control and diabetic rats. Comparison between the effectiveness of duodenal and colon mucosa. Diabetologia 37: 119-126.
33. Ziv E, Lior O, Kidron M (1987) Absorption of proteins via the intestinal wall, a quantitative model. Biochem Pharmacol 36: 1035-1039.
34. Ziv E, Bendayan M (2000) Intestinal absorption of peptides through the enterocytes. Microsc Res Techn 49: 346-352.
35. Cammisotto PG, Gelinas Y, Deshaies Y, Bukowiecki LJ (2005) Effects of insulin, glycolytic substrates and amino acids on leptin secretion from white adipocytes. Am J Physiol Metab Endo 289:166-171.
36. Cammisotto PG, Bendayan M (2007) Leptin secretion by white adipose tissue and gastric mucosa. Histo Histopathol 22: 199-210.
37. Cammisotto PG, Bendayan M (2012) A review on gastric leptin: the exocrine secretion of a gastric hormone. Anat Cell Biol 45: 1-16.