ApoA-1 mimetic restores adiponectin expression and insulin sensitivity independent of changes in body weight in female obese mice

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BACKGROUND: We examined the ability of the apolipoprotein AI mimetic peptide L-4F to improve the metabolic state of female and male ob mice and the mechanisms involved.

METHODS: Female and male lean and obese (ob) mice were administered L-4F or vehicle for 6 weeks. Body weight was measured weekly. Fat distribution, serum cytokines and markers of cardiovascular dysfunction were determined at the end of treatment.

RESULTS: L-4F significantly decreased serum interleukin (IL)-6, tumor necrosis factor-α and IL-1β. L-4F improved vascular function, and increased serum adiponectin levels and insulin sensitivity compared with untreated mice. In addition, L-4F treatment increased heme oxygenase (HO)-1, pAKT and pAMPK levels in kidneys of ob animals. pAKT and pAMPK levels were significantly reduced in the presence of an HO inhibitor. Interestingly, L4F did not alter body weight in female mice, but caused a significant reduction in males.

CONCLUSIONS: L-4F treatments reduced cardiovascular risk factors and improved insulin sensitivity in female ob mice independent of body fat changes. Reduced inflammatory cytokine levels accompanied by increased HO activity, serum adiponectin and improved insulin sensitivity suggest that L-4F may promote the conversion of visceral fat to a healthier metabolism.

INTRODUCTION

Obesity affects over 72 million adults in the United States with a disproportionate prevalence in women.1,2 Moderate to severe obesity is associated with increased risk for cardiovascular complications and insulin resistance in humans3,4 and animals.5 In particular, intra-abdominal fat correlates with insulin resistance and is an important determinant of cardiovascular risk.6 Although men have received much attention for exhibiting such ‘android’ obesity, excess visceral fat deposition is present in 41% of women in Western countries (www.dh.gov.uk).

Visceral obesity creates a state of low-grade inflammation characterized by increased pro-inflammatory cytokine levels7-9 that contributes to insulin resistance10 and oxidative stress that impairs vascular and cardiac function.5 Indeed, mice lacking tumor necrosis factor (TNF)-α have significantly improved insulin sensitivity and glucose homeostasis in response to diet-induced obesity.11-13 In addition, ob mice overexpressing adiponectin have reduced inflammation and improved insulin sensitivity, accompanied by an increase in adipocyte number and smaller adipocyte size.14 These data suggest that promoting the expansion of adipocyte number may prevent or stave off the negative inflammatory effects of larger adipocytes. The increase in inflammatory cytokine production observed with obesity may result from an increase in reactive oxygen species.15 It has been reported that reactive oxygen species produced by adipocytes increases the expression of MCP-1, a chemoattractant for macrophages, a key producer of such cytokines.5 This increase in reactive oxygen species contributes to progressive deterioration in vascular function.5

Abnormal high-density lipoprotein cholesterol (HDL-c) metabolism may also contribute to the increased cardiovascular disease risk caused by diabetes and obesity. The HDL-c particle distribution is abnormal in both types 1 and 2 diabetes, with decreases in the relative fraction of the large HDL-c particles believed to be cardioprotective.16 Apolipoprotein A-I (apoA-I) is the major protein component of HDL-c in plasma. The level of apoA-I is more closely correlated with the reduced risk of atherosclerosis than any other marker of HDL-c.17 ApoA-I prevents the oxidation and aggregation of pro-atherogenic low-density lipoprotein cholesterol (LDL-c) particles within the arterial wall and stimulates the mobilization of cholesterol from the same source.18 Infusion of apoA-I halts the progression of atherosclerosis and promotes its regression, reverses endothelial dysfunction, and induces lipid and macrophage efflux from established lesions in animals.19-21
Much effort has gone into designing peptide analogs of apoA-I that can promote the formation of HDL-c-like particles with improved potency or efficacy *in vivo*. L-4F, an apoA-1 mimic peptide, improves insulin resistance in male ob mice, reduces inflammatory cytokines, reestablishes nitric oxide (NO)/superoxide ratios and reduces consequent cardiovascular risk. D-4F, reduces atherosclerotic lesions, causes HDL-c to become anti-inflammatory, stimulates HDL-c-mediated cholesterol efflux and increases reverse cholesterol transport from macrophages. In a phase 1 human trial, a single oral dose of D-4F improved HDL-c anti-inflammatory activity. D-4F has also been shown to reduce the size of white adipose tissue stores (but not body weight) in male mice fed with a high-fat diet, and to decrease visceral fat in male ob mice. L-4F is currently in clinical trials and has been hailed as being especially promising as a potential treatment for obesity and the metabolic syndrome. However, the actions of L-4F in female ob mice in addition to the efficacy of L-4F in improving markers of vascular function in obese mice remain to be examined.

Heme oxygenase 1 (HO-1) induction is known to suppress ROS production and promote increases in insulin sensitivity. In this study, we evaluated the mechanism by which L-4F affected HO-1 and subsequently, visceral and subcutaneous fat distribution in female and male ob mice. We then examined whether reduced visceral obesity is essential for the improved metabolic profile seen in L-4F-treated animals. Male and female ob mice treated with L-4F had significant improvements in insulin sensitivity and reduced inflammatory cytokine levels. However, we show for the first time that administration of L-4F in ob female mice decreases inflammatory cytokines and improves insulin responsiveness independent of changes in body weight. Furthermore, L-4F was effective in restoring HO-1 dependent increases in NO levels in female ob mice, indicating improved vascular function, and restored superoxide production and systolic blood pressure in male ob mice. Last, L-4F treatment resulted in a marked increase in heme oxygenase (HO) activity in both male and female ob mice. Therefore, L-4F has the potential to have a crucial therapeutic role in improving metabolic syndrome parameters and indices of vascular function-associated poor cardiovascular health.

**MATERIALS AND METHODS**

**Animal care and L-4F administration**

Male and female ob mice (B6v-Lep ob/J) were purchased from Harlan (Chicago, IL, USA) at the age of 6 weeks. Lean mice (age-matched B6 V, lean, Harlan) were used as control. Sex-matched lean and ob mice were fed a normal laboratory animal diet (Research diets, New Brunswick, NJ, USA) and had free access to water. Body weight of ob and lean mice were 34±5 g and 26±3 g, respectively, and glucose levels were 229±21 mg dl⁻¹ and 154±9 mg dl⁻¹, respectively, at the start of the experiments.

At 7 weeks of age, L-4F (2 mg kg⁻¹ per day) or vehicle (ABCT: ammonium bicarbonate buffer at pH 7.4 containing 0.01% Tween-20), was administered intraperitoneally daily for 6 weeks to male and female ob and lean control mice. There were 10 groups of animals: (A) male lean, (B) male lean-L-4F, (C) male ob, (D) male ob-L-4F, (E) male ob-L-4F + SnMP (stannous mesoprophyrin was given intraperitoneally 2 mg per 100 g body weight three times per week), (F) female lean, (G) female lean-L-4F, (H) female ob, (I) female ob-L-4F and (J) female ob-L-4F + SnMP. Systolic blood pressure was determined weekly by the tail-cuff method as previously described. The Animal Care and Use Committee of New York Medical College approved all experiments.

**Effect on body weight, appearance and fat content**

At the time of killing, the body weight of all mice was measured. The subcutaneous and visceral fat visible in the abdomen, mesenteric fat, fat around the liver, kidney, spleen, heart, ovaries and testes were dissected free, pooled for each mouse and weighed. Subcutaneous and visceral fat were weighed separately.

Glucose levels and insulin tolerance tests

Mouse blood glucose was determined by testing 5 μl of tail-vein blood using an Accu-Chek (Roche, Indianapolis, IN, USA) active blood glucose-monitoring system. After a 12-h fast, mice were injected intraperitoneally with insulin (2.0 units kg⁻¹). Blood samples were taken at 0 (basal), 30, 60, 75 and 90 min, and used to measure glucose levels.

**LDL/VLDL and cytokine measurements**

Serum LDL/VLDL (very low-density lipoprotein) cholesterol levels were measured in serum collected at the time of killing (24-h post previous injection) using LDL/VLDL Quantification Kits (Biovision, Mountainview, CA, USA). Adiponectin (high molecular weight), TNF-α, interleukin (IL)-1β and IL-6 were determined in serum using an enzyme-linked immuno-sorbent assay (Pierce Biotechnology, Woburn, MA, USA). The assays were performed according to the manufacturer’s guidelines.

Measurement of cardiac superoxide (O₂⁻) levels

The hearts were placed in plastic scintillation minivials, containing 5 μmol l⁻¹ lucigenin for the detection of O₂⁻ in a final volume of 1 ml of air-equilibrated Krebs solution buffered with 10 mmol M⁻¹ HEPES–NaOH (pH 7.4) as previously described. Lucigenin chemiluminescence was measured in a liquid scintillation counter (LS6000IC, Beckman Instruments, San Diego, CA, USA).

**Determination of HO activity and NO levels**

Aortic HO activity was assayed as described previously. Billirubin, the end product of heme degradation, was extracted from chloroform, and its concentration was determined using the difference in absorbance between λ460 and λ530 nm with an absorption coefficient of 40·min⁻¹·cm⁻¹ (dual UV/VIS beam spectrophotometer lambda 2S; PerkinElmer Life and Analytical Sciences, Wellesley, MA, USA). Under these conditions, HO activity was linear with protein concentration, time-dependent and substrate-dependent.

For NO levels in whole kidney and aorta homogenate samples, 0.3 mg of protein were evaluated by measuring total nitrite and nitrate content in culture medium using the NO quantification kit and following the manufacturer’s instructions (Active Motif, Carlsbad, CA, USA).

Western blot analysis of the kidney and aorta for HO-1, pAMPK and pAkt expression

At the time of killing, aorta and kidney were harvested, drained of blood and flash frozen in liquid nitrogen. Frozen aorta and kidney segments were pulverized and placed in a homogenization buffer as previously described. Homogenates (20–50 μg of protein) were examined by protein immunoblot. HO-1, HO-2, AMPK, pAMPK, AKT and pAkt (Cell Signalling, Danvers, MA, USA) levels were determined as previously described.

**Statistical analyses**

Statistical significance was determined by the Fisher method of analysis of multiple comparisons (P < 0.05). For comparisons among treatment groups, the null hypothesis was tested by a two-factor analysis of variance for multiple groups or unpaired t-test for two groups. Data are presented as mean ± s.e.

**RESULTS**

**Effects of L-4F on male and female body fat**

L-4F treatment daily for 6 weeks prevented weight gain in ob male mice in accordance with previous findings (Figure 1a). Unexpectedly, ob female mice that received L-4F continued to gain weight at a rate similar to untreated ob mice (Figure 1a). Food intake in the male and female ob control and treated groups were comparable (ob male = 3.66 ± 0.35 g per day; ob male + L-4F = 3.6 ± 0.2 g per day; ob female = 3.5 ± 0.26 g per day; and ob female + L-4F = 3.55 ± 0.1 g per day). At the end of the 6-week treatment period, L-4F slightly decreased the ratio of...
subcutaneous fat to whole body weight in both males and females (Figure 1b). However, the ratio of visceral fat to whole body weight was decreased only in L-4F-treated males (Figure 1c). Female mice treated with L-4F showed a small but significant increase in the visceral fat to body weight ratio compared with controls (Figure 1c). The changes we measured in the fat to body weight ratio are consistent with the changes that were measured in body weight, which suggests that L-4F treatment may indeed cause sexually dimorphic responses.

Effects of L-4F on glucose levels and insulin tolerance tests
Chronic L-4F treatment reduced high fasting plasma glucose levels in ob males and females (Figure 2a). In addition, insulin administration to the L-4F-treated ob female mice resulted in a significant suppression of blood glucose levels but not in ob females receiving vehicle alone (Figure 2b). Last, the area under the curve determined for the data presented in Figure 2b was higher in vehicle compared with L-4F-treated ob females (Figure 2c). These results suggest that L-4F restores insulin sensitivity in female ob mice independent of changes in body weight (Figure 1a).

Effects of L-4F on adiponectin and cytokine levels
It has been shown that overexpression of adiponectin in the ob mouse model leads to expansion of adipose tissue. We have previously shown that L-4F increases adiponectin levels in male mice. We therefore examined whether male and female mice show a difference in adiponectin responses to L-4F. L-4F produced a significant increase in plasma adiponectin levels in both ob female and male animals (Figure 3a). However, adiponectin levels in ob female mice were increased compared with ob male mice treated with L-4F, suggesting that adipose tissue maintenance and expansion in ob female mice may result from increased adiponectin levels.

We also examined circulating levels of cytokines that are typically associated with obesity-mediated insulin resistance and vascular dysfunction. Female ob animals initially exhibited increased plasma IL-6 levels when compared with age-matched ob male mice (Figure 3b). L-4F produced a decrease in plasma IL-6 levels in both ob female and male mice when compared with untreated controls. Similar results were observed for plasma TNF-α and IL-1β levels (Figures 3c and d). Thus, although visceral fat is not lost in the female ob mice treated with L-4F, the inflammatory cytokine state is markedly improved, suggesting that the adipose tissue is healthier.

Effects of L-4F on blood pressure and vascular function
In addition to metabolic defects, ob mice have altered cardiac function, which is a common consequence of obesity. Figure 4a shows that ob male mice are hypertensive compared with lean controls. L-4F treatment was highly successful in restoring systolic blood pressure to levels matching lean controls (Figure 4a). Additionally, ob male mice had significantly elevated cardiac superoxide levels, an indicator of oxidative stress (Figure 4b). Following L-4F treatment, superoxide production was normalized (Figure 4b). Aortic NO contributes to endothelial function and protects against hypertension. NO release from the aorta of ob female mice was reduced compared with lean female mice (Figure 4c). This decrease in NO was ameliorated by L-4F treatment (Figure 4c). These results indicate that L-4F may have a role in vascular protection in obese mice.

Mice homozygous for the obese spontaneous mutation (ob mice) have elevated circulating free fatty acids, raised triglycerides, an increase in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and marked hepatic steatosis. In these mice, L-4F was effective in reducing the LDL/VLDL cholesterol ratio in both ob males and females (Figure 4e).

Effects of L-4F on HO-1 expression and HO activity
Obesity has been linked with increases in adipocyte oxidative stress, while HO-1 is strongly induced by oxidant stress and helps
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Protect against oxidative insult in cardiovascular disease. Given that we have previously shown that administration of an HO-1 inducer increases serum adiponectin levels, we wanted to examine the possibility that L-4F increases adiponectin levels by raising HO-1 levels. Thus, we examined HO-1 levels in the kidneys and aortas of ob and lean mice. As in males, HO-1 in both the aortas (data not shown) and kidneys of ob females differed significantly from lean animals (Figures 5a and b). In addition, HO-1 levels were significantly lower in ob females compared with ob males (Figure 5b). We next compared the effect of L-4F on male and female HO-1 protein expression in renal and vascular tissue samples. L-4F increased HO-1 expression in both ob male and female animals (Figure 5c). In contrast to the effects on HO-1, HO-2 levels in renal and vascular tissue samples were unaffected.

Figure 2. (a-c) Fasting glucose and insulin tolerance test. (a) Effect of L-4F treatment on glucose levels in ob male and female mice. The results are expressed as means ± s.e.m., n = 4. Levels of significance: *P < 0.01 vs ob male and #P < 0.05 vs ob female. (b) Intraperitoneal insulin tolerance test (IPGTT). The results are expressed as means ± s.e.m., n = 4. Levels of significance: *P < 0.01 and **P < 0.001 vs ob female control. (c) Area under the curve for IPGTT. The results are expressed as means ± s.e.m., n = 4. Levels of significance: **P < 0.005 vs ob female control.

Figure 3. (a-d) Effect of L-4F on serum cytokines. L-4F was administered daily for 6 weeks and serum samples were obtained immediately before killing. (a) Effect of L-4F on serum adiponectin levels in ob male and female mice. The results are expressed as means ± s.e., n = 8 - 10. Levels of significance: *P < 0.001 vs ob male, #P < 0.05 vs ob male and **P < 0.05 vs ob female. (b) Effect of L-4F on serum IL-6 levels in ob male and female mice. Levels of significance: *P < 0.01 vs ob male, #P < 0.05 vs ob male and **P < 0.05 vs ob female. (c) Effect of L-4F on serum TNF-α levels in ob mice. Levels of significance: *P < 0.01 vs ob male and #P < 0.05 vs ob female. (d) Effect of L-4F on IL-1β serum levels in ob female mice. Levels of significance: *P < 0.01 vs ob male and #P < 0.05 vs ob female.
by L-4F treatment in both male and female lean and ob animals (data not shown). L-4F increased HO activity in the aortic tissue samples of both ob male and female mice (Figure 5d). The increase in HO activity was inhibited by SnMP, an inhibitor of HO activity (Figure 5d). These results indicate that L-4F is an inducer of HO activity. Thus, the improvements in inflammatory cytokines and indices of cardiovascular function following L-4F treatment are likely mediated by L-4F-induced increases in HO activity.

To examine the contribution of vascular and renal AKT and AMPK pathways to the increase in HO-1 expression, we assessed vascular and renal expression of phosphorylated AMPK and AKT in L-4F-treated ob males and ob females. As seen in Figure 6, L-4F significantly increased basal renal AKT and AMPK phosphorylation in both ob males and females. Similar results were obtained with aortic tissue (data not shown). The addition of SnMP, a potent inhibitor of HO activity, to L-4F-treated animals reversed this effect in both ob males and females (Figures 6a-d). These results suggest that L-4F stimulation of AMPK and AKT pathways is involved in its ability to increase HO-1 expression and increase HO activity.

DISCUSSION

The metabolic syndrome is typically characterized by chronic, low-grade inflammation, excessive visceral fat, insulin resistance, abnormal cholesterol levels and increased blood pressure. The present study clearly shows for the first time that administration of the apoA-I mimetic L-4F ameliorates effects associated with the metabolic syndrome in female ob mice. Intriguingly, the positive effects of L-4F treatment on metabolic and vascular function in ob female mice were independent of changes in body weight. In fact, the visceral fat to body weight ratio increased in female ob mice treated with L-4F. We report that administration of L-4F to both ob female and male mice restored insulin sensitivity, increased serum adiponectin, decreased the LDL/VLDL cholesterol ratio and decreased serum pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β). Moreover, in ob male mice, L-4F improved systolic blood pressure and reduced cardiac superoxide production. In female obese mice, L4F caused an increase in HO-1-mediated NO levels. These novel findings suggest that L-4F has the potential to be a key therapeutic agent in the treatment of both vascular diseases and insulin resistance in obese patients.

We have previously shown that L-4F administration reduces fat mass in male ob mice.22,23 Recent studies suggest that this effect is mediated by enhanced energy expenditure resulting from upregulation of UCP1 expression in brown fat.16 In contrast, females treated with L-4F did not exhibit a decrease in visceral body fat over the time course measured (Figure 1c). These results are in line with the sexual dimorphism frequently observed in males and females in response to energetic challenges. Instead of primarily modulating food intake, females defend and build adipose stores by maintaining low rates of energy expenditure.35 Indeed, when overfed with a palatable high-fat diet, female rats gain more body weight than males because of a greater conservation of energy expenditure with lower activation of thermogenesis in brown adipose tissue.38,39

Several factors may contribute to the observed differences in effect on body fat stores. Kim et al.14 demonstrated that overexpression of adiponectin in the ob mouse leads to increased adipocyte number. Importantly, the adipocyte expansion in ob mice overexpressing adiponectin is associated with increases in insulin sensitivity.14 More recently, overexpression of adiponectin was shown to promote a more metabolically flexible adipose tissue and prevented hepatic lipid accumulation in mice fed with a high-fat diet.33 The roughly 1.5-fold elevation of adiponectin seen in L-4F-treated female ob mice above that seen in males could therefore promote adipose tissue expansion in ob female mice. Adipose tissue expansion in the L-4F-treated ob females is currently being investigated and could explain the increased visceral fat to body weight ratio despite increased insulin sensitivity and reduced serum TNF-α, IL-6 and IL-1β in the present study. This would suggest that L-4F treatment converts the visceral fat in female ob mice to a healthier phenotype, which may prevent ectopic lipid accumulation in the liver and skeletal muscle. In addition, gender differences in HO-1 and sex hormone levels currently being investigated and could explain the increased visceral fat to body weight ratio despite increased insulin sensitivity and reduced serum TNF-α, IL-6 and IL-1β in the present study. This would suggest that L-4F treatment converts the visceral fat in female ob mice to a healthier phenotype, which may prevent ectopic lipid accumulation in the liver and skeletal muscle. In addition, gender differences in HO-1 and sex hormone levels currently being investigated and could explain the increased visceral fat to body weight ratio despite increased insulin sensitivity and reduced serum TNF-α, IL-6 and IL-1β in the present study. This would suggest that L-4F treatment converts the visceral fat in female ob mice to a healthier phenotype, which may prevent ectopic lipid accumulation in the liver and skeletal muscle. In addition, gender differences in HO-1 and sex hormone levels currently being investigated and could explain the increased visceral fat to body weight ratio despite increased insulin sensitivity and reduced serum TNF-α, IL-6 and IL-1β in the present study. This would suggest that L-4F treatment converts the visceral fat in female ob mice to a healthier phenotype, which may prevent ectopic lipid accumulation in the liver and skeletal muscle.
to ameliorate the inflammatory cytokine and insulin resistant state of obese females.

HDL-c and apoA-1 have potent anti-inflammatory properties. During atherogenesis, cholesterol accumulation in macrophage foam cells induces inflammatory responses, apoptosis and other adverse effects. One of the major functions of HDL-c is to transport cholesterol from these foam cells to the liver for elimination in the bile. Cells are able to export excess cholesterol to apoA-1 by virtue of specialized cell membrane transporters, such as ABCA1, that belong to a superfamily of ATP-binding cassette transporters (ABCs). Mice lacking ABCA1 in all tissues or specifically in macrophages have a heightened response to treatment with the inflammatory stimulus lipopolysaccharide, including increased inflammatory cytokines in the circulation, implying that macrophage ABCA1 has an anti-inflammatory function. 4F peptides can mimic apoA-1 in removing cholesterol and phospholipids by the ABCA1 pathway. We are currently perusing more in-depth studies to uncover the direct role of the ABCA1 pathway in the effects of apoA-1 mimetic treatment. In addition to this mechanism, L-4F may reduce inflammation by inhibiting cellular expression of VCAM-1 and ICAM-1 on coronary endothelial cells, reducing CD11b expression on circulating monocytes and/or reducing CD11b-dependent adhesion of leukocytes to fibrinogen.

HO-1 is a stress response protein whose induction is associated with protection against oxidative stress with a concomitant increase in adiponectin. The current studies indicate that L-4F treatment decreases oxidative stress in female mice as previously seen in males. Indeed, HO-1 was dramatically induced in male mice to levels nearly twofold above those seen in lean animals, and female increases were even larger. These effects may underlie many of the beneficial actions of L-4F. For example, treatment of obese mice with an NADPH oxidase inhibitor reduced reactive oxygen species production in adipose tissue, attenuated the dysregulation of adipocytokines, and improved diabetes, hyperlipidemia and hepatic steatosis. It has been shown that induction of HO-1 serves as an intrinsic protective factor against atherosclerotic lesion formation, possibly inhibiting lipid peroxidation and influencing the NO pathway. Indeed, ob female mice exhibited decreased NO levels and elevated LDL/VLDL cholesterol ratio compared with lean female mice. Additionally, ob male mice had elevated superoxide production. These findings are in line with previous data inducting that oxidative stress decreases the messenger RNA and protein expression of ABCA1 and cholesterol efflux. L-4F administration, however, improved NO levels while decreasing the LDL/VLDL cholesterol ratio in ob female mice, and reduced superoxide production in ob male mice. Increases in HO-1 expression and HO

Figure 5. (a-d) Effects of L-4F on levels of HO-1 and HO activity. Kidney samples were subjected to western blotting for the determination of HO-1 protein expression and densitometry analysis of HO-1/actin ratio. (a, b) Expression of HO-1 in the kidney of lean and ob male and female mice. Results are means ± s.e., n = 4. Levels of significance: *P < 0.05 vs ob male. (c) Effect of L-4F on HO-1 expression in the kidneys of ob female and male mice. Results are means ± s.e., n = 4. Levels of significance: *P < 0.05 vs ob male, **P < 0.01 vs ob male and ***P < 0.001 vs ob female. (d) Effect of L-4F and SnMP on HO activity in the aortas of ob male and female mice. Results are means ± s.e., n = 4. Levels of significance: *P < 0.001 vs ob male, **P < 0.001 vs ob male + L-4F, ***P < 0.001 vs ob female and #P < 0.01 vs ob female + L-4F.
activity have also been shown to suppress inflammatory cytokine production in adipose tissue.\(^{22}\) Thus, HO-1 actions may also account for the reduction in plasma concentration of TNF-\(\alpha\), IL-6 and IL-1\(\beta\) in L-4F-treated mice.

L-4F-treated females also showed a remarkable improvement in parameters of glucose regulation. There is mounting evidence that ABCA1 interacts can also behave like a ligand/receptor system. HDL-c initiates a calcium-sensitive signaling cascade through ABCA1 that stimulates CaMKK, which phosphorylates and activates AMPK.\(^{57,58}\) Thus, HDL-c has been shown to increase muscular glucose uptake in patients with type 2 diabetes mellitus by activating AMPK-activated protein kinase in skeletal muscle in an ABCA1-dependent manner. The current studies confirm L-4F-induced phosphorylation of not only AMPK but also AKT in the kidney, suggesting L-4F can directly modulate insulin signaling and thereby improve glucose regulation. Finally, reductions in inflammatory cytokines and increases in adiponectin may also have a role in modulating insulin sensitivity and restoring glucose tolerance.\(^{7,9,35-60}\)

In conclusion, we have demonstrated that targeting the apoa-l fraction of HDL-c with a mimetic not only reduces cardiovascular risk factors, but improves insulin sensitivity and vascular health in both male and female obese mice. L-4F increases pAKT and pAMPK in a manner dependent on HO activity. Furthermore, our results demonstrate that the positive effects of L-4F are independent of changes in body weight in ob female mice. We believe that the increase in visceral fat observed in female ob mice treated with L-4F is protective and may prevent the ectopic accumulation of lipids in other insulin-sensitive tissues, a hypothesis currently under investigation. Indeed, despite an increased visceral fat, L-4F increased insulin sensitivity, reduced inflammatory cytokine levels and increased adiponectin levels.

In addition, overexpression of adiponectin in obese mice promotes adipose tissue expansion, improved insulin and glucose tolerance and reduced hepatic lipid accumulation.\(^{35}\) If extrapolated to humans, these results suggest that L-4F offers a powerful pharmacological method of treating the metabolic syndrome that may compliment weight loss therapy. L-4F appears to be a promising therapeutic strategy for treating both cardiovascular disease and insulin resistance in obese patients of either gender.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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