IL-18 Production from the NLRP1 Inflammasome Prevents Obesity and Metabolic Syndrome

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SUMMARY

Interleukin-18 (IL-18) is activated by Caspase-1 in inflammasome complexes and has anti-obesity effects; however, it is not known which inflammasome regulates this process. We found that mice lacking the NLRP1 inflammasome phenocopy mice lacking IL-18, with spontaneous obesity due to intrinsic lipid accumulation. This is exacerbated when the mice are fed a high-fat diet (HFD) or a high-protein diet, but not when mice are fed a HFD with low energy density (high fiber). Furthermore, mice with an activating mutation in NLRP1, and hence increased IL-18, have decreased adiposity and are resistant to diet-induced metabolic dysfunction. Feeding these mice a HFD further increased plasma IL-18 concentrations and strikingly resulted in loss of adipose tissue mass and fatal cachexia, which could be prevented by genetic deletion of IL-18. Thus, NLRP1 is an innate immune sensor that functions in the context of metabolic stress to produce IL-18, preventing obesity and metabolic syndrome.

INTRODUCTION

A primary function of cytokines is to initiate immune responses; however, it has become increasingly apparent that they play major roles in the regulation of energy homeostasis, implicating them in the etiology of metabolic disease (Febbraio, 2014). One such cytokine is interleukin-18 (IL-18). Obese individuals have increased levels of serum IL-18 that correlate strongly with metabolic syndrome and insulin resistance (Esposito et al., 2002; Hung et al., 2005). However, somewhat paradoxically, IL-18 has profound anti-obesity effects, demonstrated convincingly in mice where administration of IL-18 prevents weight gain, and loss of IL-18 results in exacerbated adiposity and insulin resistance by 6 months of age (Neetea et al., 2006; Zorrilla et al., 2007). It was initially proposed that mice lacking IL-18 have increased appetite and reduced energy expenditure, but recent evidence suggests that IL-18 acts to trigger AMP-activated protein kinase (AMPK) signaling and lipid oxidation in skeletal muscle, which can balance lipid accumulation on a high-fat diet (HFD) (Lindegaard et al., 2013). Therefore, in the progression toward obesity, there is continual production of IL-18 to oppose adipose tissue accumulation. It is suggested that this tonic increase in IL-18 production has the negative side effect of tolerizing the signaling pathway by decreasing IL-18 receptor expression (Zilverschoon et al., 2008). This could help to explain why obese individuals and patients with type 2 diabetes (T2D) often have very high levels of serum IL-18 but are less able to sense its beneficial metabolic effects.

The trigger that leads to increased circulating IL-18 in individuals with metabolic syndrome has not been discovered. Initially it was thought to be a byproduct of increased fat mass; however, this correlation was not reproducible (Fischer et al., 2005; Hung et al., 2005). Instead, some studies show that plasma IL-18 levels correlate with triglyceride levels (Trøseid et al., 2009; Vilarrasa et al., 2007) and that IL-18 is increased in the circulation shortly after a high-fat meal, but not after a high-carbohydrate meal.
In contrast, serum IL-18 is decreased by caloric restriction, either by diet or bariatric surgery (Schernthaner et al., 2006; Tajik et al., 2013). The cellular origin of IL-18 also remains enigmatic, although it has been shown that IL-18 released from adipose tissue beds is not produced by adipocytes themselves (Fain et al., 2006).

IL-18 is transcribed as a zymogen that requires cleavage into a mature, active cytokine that is then secreted from the cell. This cleavage is performed by the enzyme Caspase-1, when homodimerized in an intracellular protein complex known as the inflammasome. The inflammasome is oligomerized by one of a family of Nod-like receptors (NLRs), many of which operate using the adaptor ASC to link with Caspase-1. These include NLRP3, NLRP6, and NLRP12. Mice deficient for each of these NLRs have been studied; however, none of them are spontaneously obese. Mice lacking NLRP3 are in fact resistant to the diabetogenic effects of a HFD (Vandanmagsar et al., 2011). NLRP1, on the other hand, does not require ASC to activate Caspase-1 and has not been extensively studied as mice lacking this gene were only recently generated (Masters et al., 2012).

Several triggers for NLRP1 have been reported in the literature. Anthrax lethal toxin is known to activate a specific mouse allele of NLRP1 (Nlrp1b), but human NLRP1 does not respond in the same way (Boyden and Dietrich, 2006). We and others have also reported that NLRP1 can recognize Toxoplasma infection (Gorfu et al., 2014; Witola et al., 2011); however, it is unclear whether NLRP1 interacts directly with the pathogen itself. We have also shown that NLRP1 is activated in the context of hematopoietic stress such as chemotherapy or severe infection; however, again a direct NLRP1 ligand was not identified (Masters et al., 2012). The alternative is that NLRP1 functions akin to NLRP3, as a sensor of an intracellular homeostatic parameter, that when disturbed, triggers the formation of the inflammasome. This is an elegant way to propagate an inflammatory response in the context of a loss of cellular homeostasis.

We here show that mice lacking NLRP1 develop a large increase in adipose tissue spontaneously and that this is exacerbated by high-fat feeding. This increase does not occur when Nlrp1−/− mice are fed a high-fat, restricted-energy diet (HFRED). Additionally, NLRP1-deficient mice inappropriately regulate blood glucose, insulin, and leptin levels, which mirror the metabolic syndrome observed in mice lacking IL-18. All of these abnormalities are reversed in mice with an activating mutation in NLRP1, where the anti-obesity, anti-glucose intolerant effects are abolished by genetic deletion of IL-18. Profoundly, the IL-18 increase due to a HFD is fatal for NLRP1 activating mutation mice, with a marked loss of adipose tissue and cachexia. Therefore, we have documented that NLRP1 functions in the context of increased energy intake to produce IL-18 as a negative feedback signal that prevents obesity and metabolic syndrome.

RESULTS

Loss of NLRP1 Leads to Obesity

Mice lacking all three alleles of NLRP1 (Nlrp1a−/−, Nlrp1b−/−, Nlrp1c−/−; referred to as Nlrp1−/−) develop spontaneous obesity (Figure 1A), irrespective of gender (Figure S1A). This represents an increase in the fat mass while the lean mass remains unchanged, as shown in young adult mice (12 weeks old) measured by EchoMRI (Figures 1B and S1B). Pathologic examination
revealed that the source of this increased body weight was mostly due to an expansion of the visceral adipose tissue (VAT) (Figure S1C). Adipocyte size was quantified from histology, and a significant increase in adipocyte size, driven by a shift toward excess larger and fewer smaller adipocytes, was observed in Nlrp1<sup>−/−</sup> mice (Figures 1C and S1D). The subcutaneous adipose tissue (SAT) trended toward, but was not significantly expanded, and the size/mass of all other organs was unaltered (Figure 1D). Consistent with this, a bead array analysis of fasting mouse plasma revealed a significant increase in leptin for NLRP1-deficient mice (Figure 1E), while other obesity-related hormones were not altered (Figure S1E). Increased leptin is a common finding in obesity and suggests that the mice have become leptin resistant (Myers et al., 2010).

Mice lacking NLRP3 and NLRP6 are reported to have a microbiome dysbiosis that is transferable to co-housed littermate mice (Henao-Mejia et al., 2012). We co-housed WT and NLRP1-deficient mice from weaning; however, a large difference in weight was still observed, showing that this phenotype is not due to a transferrable microbial dysbiosis (Figure S1F). The obesity phenotype of NLRP1-deficient mice was not due to hyperphagia, as they consumed the same amount as WT mice (Figure S1G), and the day/night time activity of the mice was also not altered (Figure S1H). Instead, there was a significant decrease in the average respiratory exchange ratio over a 24-hr period (RER VCO₂/VO₂), although this did not reach significance for the day or night periods by themselves (Figure 1F). This was associated with increased heat production by the mice (Figure 1G). This reveals a metabolic shift in the Nlrp1<sup>−/−</sup> mice toward lipid utilization (i.e., decreased RER) and heat production, consistent with increased adiposity. Given the large increase in adiposity in these mice and previous reports that mice deficient in IL-18 suffer from a deficit of lipid oxidation (Lindegaard et al., 2013), we measured total triacylglycerol (TAG) in two hind-limb skeletal muscles. Consistently, this was increased in NLRP1-deficient mice compared with control (Figure 1H). These data show that loss of NLRP1 recapitulates the obesity phenotype of mice lacking IL-18 via an intrinsic metabolic mechanism.

**Loss of NLRP1 Leads to Metabolic Syndrome**

The increased deposition of TAG in muscle can prevent appropriate clearance of glucose from the circulation and is associated with metabolic syndrome (Kelley and Goodpaster, 2001). Consistent with this idea, we found a defect in the glucose clearance from the blood of 8- to 12-week-old Nlrp1<sup>−/−</sup> mice during an intraperitoneal glucose tolerance test (IPGTT) (Figure 2A). This was more pronounced in older, more obese mice (Figure S2A). An oral glucose tolerance test (OGTT) has the advantage of incorporating the incretin axis, and again, Nlrp1<sup>−/−</sup> mice do not appropriately clear blood glucose when dosed via this route (Figure 2B). Consistent with the glucose intolerance in the Nlrp1<sup>−/−</sup> mice, we also observed significantly higher non-esterified fatty acids (NEFAs) at the 15-min time point of the OGTT, indicating a lack of sensitivity of the adipose tissue to suppress lipolysis in response to insulin (Figure 2C). In contrast, insulin production was not altered over this timeframe (Figure 2D), and pancreas histology (islet size) was normal (Figure S2B). Therefore, along with obesity, mice lacking NLRP1 also share the same metabolic defect as mice lacking IL-18, attributable to a failure of glucose clearance, rather than impaired insulin production.

**HFD Exacerbates Obesity, Metabolic Syndrome, and Steatosis in Nlrp1<sup>−/−</sup> Mice**

We next fed 6-week-old male WT and Nlrp1<sup>−/−</sup> mice a HFD (60% energy from lipids, 22.8 MJ/kg, 50.1 KJ/mouse/day) for 8 weeks and examined growth curves. The Nlrp1<sup>−/−</sup> mice rapidly gained weight compared with the WT controls (Figure 3A), and we also noted that the increase in body weight was more pronounced than for Nlrp1<sup>−/−</sup> mice fed regular chow (26% energy from lipid, 13.2 MJ/kg, 39.6 KJ/mouse/day). HFD-fed Nlrp1<sup>−/−</sup> mice had a significant increase in VAT, SAT, and liver weights (Figures 3B and S3A). Brown adipose tissue (BAT) weight was not increased (data not shown). H&E staining of the liver revealed extensive steatosis as indicated by widespread vacuoles (Figure 3C). As for mice fed a regular chow diet, loss of NLRP1 did not affect food intake of the HFD (Figure 3D), and the RER was significantly increased.
decreased, this time during all periods of the day (Figure 3E). Commensurate with increased adiposity, adipocyte size itself was increased (Figures 3F, 3G, and S3B), and plasma leptin levels were elevated (Figure 3H). Since there was a significant increase in adipocyte size, suggesting increased lipid storage, we next examined NEFAs during a feeding (0 hr) and fasting (12 hr) state. This revealed a significant defect in lipolysis when the system was stressed on the HFD, which was greatly exacerbated after starvation (Figure 3I). Alongside NEFAs, lipolysis also liberates glycerol into the circulation, and we could confirm that this
was significantly decreased in fasted Nlrp1<sup>1−/−</sup> mice (Figure 3J). Next, HFD-fed mice were subjected to an IPGTT, which revealed a major defect in the ability for the Nlrp1<sup>1−/−</sup> mice to clear blood glucose (Figure 3K). This was accompanied by a significant elevation in fasting plasma insulin levels in the Nlrp1<sup>1−/−</sup> mice (Figure 3L), which taken together suggests that these mice are insulin resistant. Finally, we performed western blotting for mature IL-18 in the VAT after HFD (Figure 3M), which revealed a significant decrease in the Nlrp1<sup>1−/−</sup> mice (Figure S3C) and provides more evidence that this is the key inflammasome that produces IL-18 during obesity.

**Obesity Regulated by Non-hematopoietic Expression of NLRP1**

To help identify the cell type in which NLRP1 is active, we generated bone marrow chimeras, in which WT or Nlrp1<sup>1−/−</sup> mice were lethally irradiated at 6 weeks of age, and reconstituted with bone marrow from WT or Nlrp1<sup>1−/−</sup> donors. At 12-weeks post-transplant, they were placed on HFD. Weekly weighing indicated that while Nlrp1<sup>1−/−</sup> recipients of either WT or Nlrp1<sup>1−/−</sup> bone marrow developed obesity, WT recipients did not (Figure 4A). This was also evident upon autopsy after 12-weeks HFD, where VAT and SAT weights were significantly increased in mice where Nlrp1 was deleted in non-hematopoietic cells (Figure 4B). Bone marrow chimeric mice were also subjected to an IPGTT, which agreed with the previous results, that NLRP1 functions in non-hematopoietic cells to render mice less able to clear glucose from circulation over time (Figure 4C). These data show that deletion of NLRP1 from non-hematopoietic cells leads to adiposity and glucose intolerance, which is a clear contrast to NLRP3 and ASC, which are reported to function in bone marrow-derived cells during HFD (Wen et al., 2011).

**NLRP1 Functions in Response to High-Energy, Not High-Fat, Feeding**

To test whether lipids or lipoproteins derived from a HFD triggered the NLRP1 inflammasome, we compared this to a diet that was similarly high in fat (75% energy from lipids), but with a reduced overall energy density (12.7 MJ/kg, 43.4 KJ/mouse/day). Interestingly, this HFRED diet reversed the phenotype of these mice, and although the mice increase their weight with age, this was no longer different to WT mice (Figure 5A), with no increase in adipose tissue or organ weight (Figure 5B), Next, mice fed this alternate diet were subjected to an IPGTT, which confirmed that the HFRED prevented development of metabolic syndrome in mice lacking NLRP1 (Figure 5C). This is also in agreement with no difference for plasma insulin and leptin levels (Figure 5D).

As opposed to regular chow, where the principle energy source is carbohydrate, and HFD, where the principle energy source is lipid, we next fed mice a diet where the principle energy source was protein (60% energy from protein, 17.7 MJ/kg, 40.7 KJ/mouse/day). This high-protein diet (HPD) led to significant weight gain in the mice (Figure 5E), which was evident in the VAT, SAT, and the liver (Figure 5F). Mice fed HPD for 8 weeks were glucose intolerant during an IPGTT (Figure 5G), and although starved plasma insulin levels were not different, a significant increase in leptin was observed (Figure 5H). Together these data suggest that it is the energy quotient of the diet, rather than source of the energy, that contributes to obesity in NLRP1<sup>1−/−</sup> deficient mice.

**Analysis of Obesity and Metabolic Syndrome in Nlrp1MUT Mice**

As a direct comparison to the effect of deleting NLRP1, we analyzed mice with an activating mutation (Q593P) in Nlrp1a (referred to here as Nlrp1<sup>MUT</sup>). These mice experience a spontaneous inflammatory disease characterized by elevated neutrophils, that is entirely driven by IL-1 (Masters et al., 2012). They also exhibit elevated plasma levels of IL-18, so we would expect that if NLRP1 induced IL-18 has anti-obesity effects then these mice should have significantly less adipose tissue compared with their controls. Consistent with this prediction, Nlrp1<sup>MUT</sup> mice weigh less than control mice and the adipose tissue mass is markedly reduced (Figure S4A). However, because these mice have a spontaneous inflammatory disease, it is possible that the decrease in fat mass is due to hyperthermia, or another aspect of the disease that is unrelated to the metabolic disturbance in Nlrp1<sup>1−/−</sup> mice. We therefore examined Nlrp1<sup>MUT</sup> mice lacking the IL-1 receptor (IL1r<sup>−/−</sup>), which have no evidence of inflammatory pathology, and have a normal lifespan (Masters et al., 2012). Again the specific decrease in fat mass was clear (Figure 6A). This experiment also had the added advantage of excluding a role for IL-1 in the anti-obesity effects of active NLRP1. Consistently, we observed no changes in food intake (Figure 6B) and collectively this definitively rules out a role for NLRP1 in satiety.
Next we placed the IL1r−/− Nlrp1MUT mice on a HFD and found that they did not accumulate increased weight or VAT compared with control mice (IL1r−/−) (Figures 6C and 6D). In agreement with this, fasting plasma leptin was vastly decreased, although insulin was not (Figures 6E and 6F). TAG levels were also the opposite to Nlrp1−/− mice, being significantly decreased in the tibialis anterior (TA) muscle (Figure 6G). Furthermore, these mice were more glucose sensitive than controls, during an IPGTT (Figure 6H). Surprisingly, after 7 weeks on the HFD, mice with an activating mutation in NLRP1 became cachexic and moribund with none surviving past 15 weeks of high-fat feeding (Figures 6I and S4B). In contrast to the steatosis that develops in control mice on a HFD, livers of IL1r−/− Nlrp1MUT mice were devoid of vacuoles, and exhibited large necrotic foci (Figure 6J). Most strikingly, elevated IL-18 in the plasma of IL1r−/− Nlrp1MUT mice was increased by more than an order of magnitude, due to the HFD (Figure 6K). This shows that IL-18 is processed by NLRP1 and when induced under the setting of increased caloric intake can produce levels of IL-18 that are ultimately fatal.

Analysis of Obesity and Metabolic Syndrome in IL1r−/−IL18−/−Nlrp1MUT Mice

To formally prove that IL-18 is the cytokine mediating the effects of NLRP1, we generated IL1r−/−IL18−/−Nlrp1MUT mice. These were compared to littermate IL1r−/−IL18−/−Nlrp1+/− mice. These had succumbed to loss of weight and cachexia (n = 6). This confirms that the mechanism by which NLRP1 regulates obesity and metabolic dysfunction is through IL-18.

Together these results document that NLRP1 is a fundamental innate immune receptor that processes IL-18 in the context of excess energy intake as a feedback mechanism to prevent weight gain and downstream consequences on peripheral metabolism.

DISCUSSION

The original finding that mice lacking IL-18 develop obesity and metabolic syndrome was a significant development for the field (Netea et al., 2006). This had direct translational implications, as IL-18 was long known to be associated with obesity and metabolic syndrome in humans. However, the mechanism leading to IL-18 production as a negative feedback signal in these conditions was not established. Here we show that mice lacking the innate immune sensor NLRP1 recapitulate all of the metabolic phenotypes of IL18−/− mice that we have interrogated so far. Moreover, mice with genetically activated NLRP1 resist obesity and metabolic syndrome due to a HFD, but not if IL-18 is removed from the system.

To establish the components of a HFD that are associated with NLRP1 activation, we fed mice a diet that remained high in lipids, but with a reduced energy density, or a diet where the primary energy source was protein. This showed that it was energy intake, rather than source of energy, that exacerbated the NLRP1 phenotype (Figure 5). One explanation for this could be that NLRP1 is directly activated by a metabolic byproduct. This would seem to be in contradiction to in vitro siRNA or overexpression studies, which show that NLRP1b is activated by IL-18.
metabolic inhibitors and nutrient deprivation (Liao and Mogridge, 2013). However, we failed to reproduce those findings using cells from genetically deficient Nlrp1−/− animals (data not shown). It was also proposed that leptin is a trigger for specific IL-18 production via Caspase-1 (Jitprasertwong et al., 2014); however, our data suggest that leptin changes in response to NLRP1 activation, rather than in reverse (Figure 6E). Alternatively, increased energy uptake may in fact upregulate pro-IL-18 levels, if NLRP1 has baseline activity that constitutively activates IL-18 in certain cell types.

The cell type in which NLRP1 functions to cleave IL-18 in this context remains an area of active investigation. Our data suggest that this is a non-hematopoietic cell (Figure 4), although some tissue resident cells such as macrophages may be radioresistant. Indeed, NLRP1 is expressed in relevant tissues, such as the WAT, which is further increased by HFD (Sun et al., 2012). Although we observed a 10-fold increase in the plasma IL-18 of Nlrp1MKO mice on a HFD (Figure 6K), we did not see a decrease in plasma IL-18 for Nlrp1−/− mice (data not shown). Importantly, we did detect a significant decrease in IL-18 by western blot of Nlrp1−/− VAT after HFD (Figure 3M). This suggests that local, tissue based IL-18 production, rather than circulating IL-18, controls the phenotype. If the effects of IL-18 are localized, it also helps to explain how obese individuals can have elevated circulating levels of this anti-obesity signal, but not benefit greatly from its effects. This also suggests that locally acting IL-18 therapies might afford weight loss benefits in obese individuals.

The mechanism by which IL-18 functions to deplete adipose tissue mass is suggested to be via activation of AMPK to increase lipid accumulation. Preliminary studies did not reveal a profound difference in the phosphorylated forms of AMPK or its target ACC in the muscle of NLRP1 deficient mice (data not shown). However, our results are consistent with a mechanism that involves a defect in lipid accumulation, as HFD-fed Nlrp1−/− mice have no increase in serum NEFAs or glycerol after fasting, but increased muscle TAG, adipose tissue mass, adipocyte size, and lipid deposition in the liver. Moreover, mice with an activating mutation in NLRP1 display the opposite phenotype and, in addition, become cachexic and moribund on a HFD. It is likely that this reflects an absence of lipid as an energy source in muscle, in concert with the depletion of lipids from adipose tissue, which is almost entirely deficient. It would be very interesting to see if a HFD is also fatal for IL-18 transgenic or IL-18bp-deficient mice.

Figure 6. Activation of NLRP1 Prevents Obesity and Metabolic Syndrome
(A and B) Tissue weight (A) and food intake (B) for control (IL1r−/−) or IL1r−/− Nlrp1MKO mice.
(C–F) Mice fed a HFD were (C) weighed weekly and after 8 weeks (D) tissue weight and (E) fasting plasma leptin or (F) insulin was measured.
(G–I) TA muscle TAG (G), IPGTT (H), and survival curves for the HFD fed mice (I).
(J) Liver histology was assessed by H&E staining.
(K) Fasting plasma IL-18 levels measured by ELISA. Analysis is displayed as means ± SD and significance determined by t test, ANOVA or t test of AUC (n = 3–6).
In order to combat the worldwide obesity epidemic, it is of fundamental importance to understand the homeostatic innate immune mechanisms that are employed by the body to try and prevent obesity, insulin resistance, and the development of T2D. Our data provide compelling evidence that the innate immune system is activated not only during infection, but also in response to the loss of cellular metabolic homeostasis associated with a high-energy diet. It is likely that NLRP1 is integrating a number of factors into the decision about how to maintain homeostasis through inflammasome activation and IL-18 production, so this may explain links between obesity/metabolic syndrome and infections/environmental insults. In summary, NLRP1 is a physiological sensor that can respond to and prevent obesity and metabolic syndrome, which are causing a dramatically increasing burden of disease throughout the world.

EXPERIMENTAL PROCEDURES

Mice

Nlrp1a-C0/Nlrp1b-C0/Nlrp1c-C0 (Nlrp1-C0) (Masters et al., 2012), Nlrp1a-Q593P/Q593P (Nlrp1MUT) (Masters et al., 2012), Il18-C0/C0 (Takeda et al., 1998), and Il1r-C0/C0 (Labow et al., 1997) mouse strains were generated on or had been backcrossed at least ten generations with the C57BL/6 background. All experiments use littermate controls or their immediate descendants. Bone marrow chimeric mice were generated by subjecting recipient mice to two 5.5-Gy doses of irradiation given 3 hr apart and then injecting 5 × 10⁶ donor bone marrow cells IV. All animal experiments complied with the regulatory standards of and were approved by the Walter and Eliza Hall Institute and the Alfred Medical Research and Education Precinct Animal Ethics Committees.

Histopathology

Organs were collected in 10% buffered formalin. Tissue sections were prepared from paraffin blocks and stained with H&E. An automated script to quantify adipocyte size was compiled using FIJI, and the distribution of sizes was determined as a percentage, for adipocytes with sizes ranging between 0 < 1,000, 1,000 < 2,000, 2,000 < 3,000, ..., x < x + 1,000 μm².

Diet

Apart from a standard chow diet, mice were fed either a HFD (60% energy from lipids), a HPD (60% energy from protein), or a high-fat, restricted-energy diet (75% energy from lipids, 51% acid detergent [AD] fiber) (Specialty Feeds). Further details are provided in Table S1. Feeding was performed ad libitum for up to 15 weeks.

EchoMRI

Mouse body composition (fat mass and lean body mass) was measured weekly with a 4-in-1 EchoMRI body composition analyzer (Columbus Instruments) with total body mass measured using standard laboratory scales.

Indirect Calorimetry and Activity

Mice were placed in a 12-chamber indirect calorimeter (Oxymax series; Columbus Instruments) with an airflow of 0.6 L/min. RER and heat production were measured using the system. Activity is measured as total beam breaks (x+y+z axis). Mice had free access to food and water while in the chambers. Food intake was measured for the duration of data collection while mice underwent the indirect calorimetry measurements.

IPGTT, OGTT

For IPGTT, all mice were fasted for 5 hr and then were injected with the same dose of 45-mg glucose, as we previously found them to have the same lean mass (Figure 1B). For OGTT, mice were fasted for 5 hr and then gavaged with 2-g/kg glucose according to lean body mass. During the IPGTT and OGTT, blood was collected for measurement of glucose, insulin, and NEFAs.

BioPlex, ELISA, Non-esterified Free Fatty Acid, Glycerol, and TAG Analysis

Insulin, leptin, and other obesity-related markers were measured using the BioPlex bead array (BioRad) according to the manufacturer’s instructions. IL-18 was analyzed by sandwich ELISA using IL-18-specific antibodies (Westwell-Roper et al., 2013). Non-esterified free fatty acids (NEFAs), glycerol, and
Tissue samples were lysed, and protein concentration was determined as previously described (Kraakman et al., 2015). Immunoblotting was performed using the following primary antibodies: IL-18 (MBL clone D047-3); HSP90 (Cell Signaling Technology).

**Western Blotting**

Tissue samples were lysed, and protein concentration was determined as previously described (Kraakman et al., 2015). Immunoblotting was performed using the following primary antibodies: IL-18 (MBL clone D047-3); HSP90 (Cell Signaling Technology).

**Statistics**

Unless otherwise specified, data are presented as mean ± SD. Comparisons were performed using a Student’s t test, ANOVA, or comparison (t test) of area under the curve (AUC). Survival curves were analyzed using a log-rank (Mantel-Cox) test.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, Figures S1–S4, and Table S1 and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.09.024.

**AUTHOR CONTRIBUTIONS**


