Nicotinamide protects against diet-induced body weight gain through increased energy expenditure and white adipose tissue beiging

Short title: Anti-obesity effects of the amide form of vitamin B3

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Highlights

NAM supplementation enhances energy metabolism in diet-induced obese mice

White adipose tissue beiging and oxidative metabolism is induced upon NAM administration

NAM administration prevents white adipose tissue inflammation

NAM supplementation ameliorates fatty liver

In brief

Méndez-Lara et al. show that NAM supplementation prevents weight gain by inducing NAD+ content and white adipose tissue beiging. An increased number

of brown and beige/brite adipocyte clusters, protein abundance of Ucp1, mitochondrial activity, adipose NAD+, EL ANALSISIS DE SENSIBILIDAD A INSULINA NO ES ESPECIFICO DE TAB and Ampk levels was observed in white adipose tissue of treated mice. Hepatic steatosis was prevented and inversely related to decreased gene expression of Cd36. The effect of NAM in diet-induced obese mice also ameliorated the inflammatory phenotype by increasing circulating levels and adipose gene expression of adiponectin and IL-10.

Abstract

Interventions that boost NAD+ availability are of potential therapeutic interest for obesity treatment. The potential of nicotinamide (NAM), the amide form of vitamin B3 and a physiological precursor of NAD+, in preventing weight gain has not previously been studied *in vivo*. Other NAD+ precursors have been shown to favorably decrease weight gain *in vivo*; however, their impact on adipose tissue was not described. Here we show that NAM supplementation protected against diet-induced obesity by augmenting global body energy expenditure. The manipulation markedly altered adipose morphology and metabolism, particularly in inguinal (i) white adipose tissue (iWAT). An increased number of brown and beige/brite adipocyte clusters, protein abundance of Ucp1, mitochondrial activity, adipose NAD+, insulin sensitivity, and induced Ampk levels was observed in iWAT of treated mice. Notably, a significant improvement in hepatic steatosis and inflammation was also observed in NAM high-dose (HD)-treated mice. These data indicate that NAM is a nutrient supplement that influences whole-body energy expenditure by driving changes in the adipose phenotype. Thus, NAM is

a novel and attractive dietary supplement for preventing obesity and associated complications.

Keywords: adiposity, beiging, fatty liver, inflammation

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1. Introduction

Obesity is a complex, chronic disease [1] and a main public health concern worldwide [2], which is defined as an imbalance of energy between energy intake (i.e, food consumption) and energy expenditure (i.e., basal metabolism, physical activity and adaptive thermogenesis) [3].

In the last two decades there has been a remarkable increase in our understanding of adipose biology. The white adipose tissue (WAT) is designed as the main long-term, energy-reservoir organ and actively controls energy homeostasis [4]. Given its energy buffering capacity and endocrine function, healthy WAT manages excess energy storage by controlling both energy intake and expenditure [4].

However, in obesity, WAT becomes severely dysfunctional and generally undergoes pathological remodeling and metabolic derangements, which triggers uncontrolled pro-inflammatory responses [5]. Compelling evidence suggest that an altered management of excess free fatty acids (FFA) by WAT results in metabolic stress and toxicity in adipocytes (lipotoxicity) and other relevant cell types, including myocytes, hepatocytes, and immune cells [4]. Systemic chronic, low-grade inflammation is another hallmark of WAT in obesity [4]. It is frequently characterized by moderate elevations in proinflammatory cytokines with concomitant reductions in anti-inflammatory cytokines, as well as infiltration and proinflammatory responses of different immune cell types, including macrophages [4]. Notably, chronic inflammation in WAT during obesity has a major impact on adipocyte metabolism and function.

Obesity is a largely preventable disease [10]. However, the effectiveness of existing pharmacological and/or dietary initiatives for long-treat treatment of

obesity failed, at least in part could be due to strategies which do not target adipose tissue, a situation that is beginning to change [10]. Pharmacological and nutritional interventions stimulating mitochondrial biogenesis and function confers protection against diet-induced obesity (DIO) [7]. In this context, the role of nicotinamide adenine dinucleotide (NAD)+ has emerged as a signaling molecule that plays a major regulatory role in many cell functions, including inflammation and energy metabolism, by modulating the action of protein such as sirtuins, which use NAD+ as a coenzyme [7, 8]. Different lines of evidence suggest that strategies leading to increasing adipose tissue NAD+ could be a target for treating obesity. For instance, cellular NAD+ decline is a DIO hallmark and induces the development of many of the ailments associated with this condition [9-15]. Conversely, tissue content of NAD+ raises in response to exercise [16-19] or calorie restriction interventions [20], both of which are interventions associated with decreasing body weight. Furthermore, supplementation with NAD+ precursors, such as nicotinamide riboside (NR) and mononucleotide nicotinamide (NMN), increased energy metabolism and prevented body weight gain by improving mitochondrial function, confering protection against metabolic DIO complications [10, 12, 21]. Despite data showing the efficacy of NAD+ precursors on adipose metabolism [15], the mechanisms accounting for the broad spectrum of their health benefits are still poorly understood. Indeed, the impact of these molecules on the biology/physiology of different adipose tissues, i.e., brown (BAT) and white adipose tissues (WAT), has not been addressed.

Nicotinamide (NAM) is also a physiological precursor of NAD+. However, its contribution in boosting energy metabolism in adipose tissue and body weight gain remains largely unknown. Although a growing body of evidences supports a

role for NAM as an anti-diabetic agent [22][14, 23] which ameliorates non-alcoholic fatty liver disease [24], its potential contribution to body weight reduction remained to be characterized. We therefore tested the hypothesis that administration of NAM in vivo protects against diet-induced obesity.

2. Materials and methods

All animal procedures were reviewed and approved by the Institutional Animal Care Committee and Use Committee of the Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau (Procedure id 10434), and the methods were conducted in accordance with the approved guidelines. The effect of NAM supplementation was conducted in male (strain C57BL/6J) mice. Two doses of NAM (high dose 1%; and low dose 0.25%) were given via drinking water to mice, starting at the same time as the high-fat diet feeding. The biochemical measures were determined using commercial kits adapted to a COBAS c501 autoanalyzer as described [25]. NAM was analyzed using high-performance liquid chromatography (HPLC) with mass spectrometry (MS). NAD+/NADH and adenosine monophosphate (AMP) in adipose tissues were determined using a commercial assay (from Abcam and Buffalo University, respectively) according to the manufacturer's instructions. The design of the intervention is shown in **Supplementary Figure 1**. Systemic concentrations of cytokines (IL10, IL6, IL4 and TNF-a) were analyzed on a Luminex using xMAP® technology (Millipore Corporation, Billerica, MA). Adipokines were measured using enzyme-linked immunosorbant assay kits (ELISA; Invitrogen). RNA samples from tissues were extracted and reverse transcribed and relative mRNA levels of each gene determined by real time qPCR. Western blots were performed following standard procedures using specific antibodies against Ucp1 and total and phosphorylated

AMP-activated protein kinase (p-AMPK), respectively. Palmitate β-oxidation measurement in isolated mitochondria was performed as described [26]. White and brown adipose tissue and hepatic biopsies were taken for histological evaluation (hematoxylin and eosin staining) in paraffin embedded sections using a light field microscope. Measurements of oxygen consumption (VO_2) and CO_2 production (VCO_2) were performed using an indirect calorimetry system (Oxymax/CLAMS, Columbus Instruments). Magnetic resonance imaging (MRI) and spectroscopy (MRS) analysis was performed using a 7T Bruker BioSpec 70/30 USR (Bruker BioSpin GmbH, Ettlingen, Germany) system. Data are mean ± SEM. Two-tailed student's-tests were used for single comparisons and analysis of variance (ANOVA) with Newman-Keuls post-hoc tests for multiple comparisons. The relationships between different variables were determined by calculating Pearson's or Spearman's correlation coefficients, as appropriate. Statistical significance was assumed if $P<0.05$. Additional details Methods are available in the online version of the paper (**Supplementary Methods**).

3. Results

NAM supplementation causes body weight gain prevention

In order to determine the effects of NAM on preventing diet-induced obesity, mice were first fed a high-fat diet and treated with two different concentrations of NAM for 12 consecutive weeks. NAM was well-tolerated by mice as shown by similar water intake rates among the different groups (**Table 1**). Plasma levels of NAM were steadily elevated in NAM treated groups (NAM LD: 6.5-fold, $P<0.05$, NAM HD: 18-fold, $P<0.05$) compared with untreated mice **(Figure 1, panel A; Table 1**). Blood chemistry panels, including tests of liver and kidney function, and urine

did not detect any sign of NAM toxicity (**Table 1, Supplementary Tables 1 and 2**).

Final body weight or volume (ME REFIERO A FIG 1 PANEL C) was significantly reduced only in mice receiving the highest dose of NAM compared with untreated mice (**Table 1 and Figure 1, panel B and C**). The average percentage of body weight prevention normalized to untreated mice was 10.2% (REVISALO, A MI ME SALE UN 19,8%) ($P<0.05$) in NAM HD-treated mice. The final body weight of mice correlated inversely with plasma NAM (Spearman $r = 0.76$, $P<0.05$) **(Supplementary Figure 2, panel A).** Body weight gain prevention was 69.5%, $P<0.05$) in NAM HD-treated mice and 35,8% in NAM LD respect to untreated mice (**Table 1**). This was mainly attributed to body fat gain prevention and, to a lesser extent, also to decreased total lean mass (**Table 1).** Consistently, NMR and fat mass depot analysis (**Figure 1, panels D, E and F**) showed similar information and total fat pad was directly associated with body weight in these mice (Spearman $r = 0.9522$, $P<0.05$ **(Supplementary Figure 2, panel B),**. In contrast, body fat fraction in NAM LD-treated mice often did not differ from untreated mice. Fat pad reduction was accompanied by a concomitant reduction (59.5%, $P<0.05$) in the cross-sectional area of adipocyte from NAM HD-treated mice (**Figure 1, panel G**).

Although plasma glucose or insulin levels in NAM-treated mice did not significantly differ from untreated mice (**Table 1)**, NAM treatment improved glucose tolerance in mice treated with the highest dose of NAM (21%, $P<0.05$) compared with untreated mice (**Figure 1, panels H and I**). No changes were observed in insulin sensitivity **(Figure 1, panels J and K)**. Plasma concentrations of FFA were significantly decreased in NAM-treated mice compared with

untreated mice, whereas total cholesterol and triglycerides did not differ among groups (**Table 1**). The decreased adiposity shown by HFD-fed animals was accompanied of an elevation in circulating adiponectin in NAM HD mice (1.4-fold, P<0.05) compared with untreated mice (**Table 1**). In contrast, plasma leptin concentrations were reduced in NAM HD mice (79.1%, P<0.05) compared with untreated mice (**Table 1**).

Histological analysis of WAT revealed the presence of characteristic crown-like structures (CLS) in untreated mice (**Figure 1, panel L**). Of note, the number of CLS was significantly diminished (85%, P<0.05) in WAT from NAM HD mice diminished (300 ± 30 CLS/10,000 adipocytes) compared with untreated mice (45 ± 8 CLS) (**Figure 1, panel L**). Consistently, two gene markers of macrophage infiltration were significantly reduced in WAT of NAM HD mice (*Cd68*: ~40%, P<0.05) compared with untreated mice (**Figure 1, panel M**). Additionally, relative mRNA levels of *Il10* in WAT of NAM-HD mice presented significant elevation (~2.7-fold, P<0.05) whereas the mRNA levels of *Tnfa* did not differ among groups (**Figure 1, panel M**). Plasma levels of several pro- and anti-inflammatory cytokines were also measured (**Figure 1, panel N**). Plasma IL-10 showed a close-to-significant trend to be increased, while plasma IL-6 was marginally decreased in NAM HD mice.

NAM administration ameliorates fatty liver

NAM did not alter liver weight (**Table 1, Figure 1, panel F**). Plasma levels of ALT and AST in NAM-treated mice did not differ either from those of untreated mice (**Supplementary Table 2**). Interestingly, NAM supplementation prevented HFD-induced hepatic steatosis (**Figure 2, panels A and B**) as seen by the decreased intracellular lipid droplets (39.9%, P<0.05) in histological preparations in NAM HD

mice) and MRS analysis -(69.5%, P<0.05), respect untreated mice. Furthermore, the liver fat fraction directly correlated to both whole-body fat volume (Spearman r = 0.9301, P<0.05) and whole-body volume (Spearman r = 0.7552, P<0.05) **(Figure 2, panels C and D).** Notably, the hepatic mRNA levels of *Cd36* and *Fgf21* and the plasma concentration of Fgf21 were reduced, especially in NAM HD mice (*Cd36*: -51%, P<0.05; *Fgf21*: -39%, P<0.05; Fgf21: (-83%, P<0.05, respectively) (**Figure 2, panel E and F**).

Effects of NAM administration on energy expenditure

Body weight gain prevention by NAM was not accompanied by significant changes in the daily food consumption of NAM-treated mice (**Table 1, Figure 3, panel A**). It is worthy to note that food intake was measured before body weights diverged to eliminate confusions by body-weight differences [11]. Additionally, fecal triglycerides did not differ among groups (NAM HD: 1.74 ± 0.43 µmol/g vs. Un: 1.52 ± 0.36 µmol/g, P=0.98. Therefore, increased energy expenditure rather than decreased food intake or steatorrhea may explain the fatless in NAM HD mice. Indeed, feed efficiency (body weight change per kcal of food eaten) was significantly reduced (84.6%, P<0.05) only in NAM HD mice (**Figure 3, panel B**). Increased energy expenditure was directly demonstrated by indirect calorimetry The 24-h VO_2 and the respiratory exchange ratio (RER) of NAM HD mice were significantly higher (VO_2: 20% during day and night (**Figure 3, panel C)**. RER in NAM HD mice was increased 2.4% during day and 3.6% during night (P<0.05) respect untreated mice (**Figure 3, panel D; Supplementary Figure 2, panels C and D**), NAM-LD treated mice only showed a significant VO_2 increase during night (VO_2: 10%, P<0.05; RER: 2.3%, P<0.05) (**Figure 3, panels C and D**). RER was inversely related to either body weight (day: Spearman r = -0.6731, P<0.05;

night: Spearman r = -0.6082, P<0.05) and fat pad (day: Spearman r = -0.5985, P<0.05; night: Spearman r = -0.5857, P<0.05) (**Supplementary Figure 2, panels E and F**). Data from energy expenditure analysis in different groups of mice were Ancova corrected prior to comparison among different group pairs (**Figure 3, panel E)**. Energy expenditure in NAM LD mice did not differ from untreated mice but was significantly increased in NAM HD mice (1.4-fold, P<0.05) compared with untreated mice (**Figure 3, panel E**). Locomotor activity did not differ among groups (**Supplementary Figure 2, panel G**).

NAM administration induces WAT beiging

Since we observed an increase in energy expenditure, NAM might promote increased iBAT activity. NAM manipulation did not influence iBAT mass (**Figure 4, panel A**). Analysis of iBAT from NAM HD showed that brown adipocytes had much less accumulation of lipid vesicles (0.6-fold, P<0.05) than untreated mice (**Figure 4, panel B**). Notably, mitochondrial fatty acid β-oxidation was elevated (1.3-fold, P<0.05) in iBAT of NAM HD mice compared with untreated mice (**Figure 4, panel C**). This finding was not associated with changes in the expression of mitochondrial genes (**Figure 4, panel D**). Similarly, the relative protein abundance of Ucp1 in iBAT of NAM HD mice did not differ from untreated mice (**Figure 4, panel E, left bar chart subpanel**). However, corrected levels of Ucp1 by total iBAT protein were significantly increased in these mice (1.9-fold, P<0.05) (**Figure 4, panel E**, **right bar chart subpanel**), suggesting iBAT hyperplasia.

Under certain conditions adipose thermogenesis may be activated and contribute to energy expenditure. Beige adipocytes, which may be found in WAT, can functionally mimic the metabolic actions of classical brown adipocytes, including

thermogenesis [27]. As inguinal (i)WAT is the subcutaneous fat depot most readily beiged in C57BL/6 mice [28, 29], we investigated whether NAM-mediated increased energy expenditure was associated with WAT beiging. NAM HD administration in mice markedly altered adipose morphology (**Figure 5, panel A**), promoting the appearance of small-sized adipocytes. The number of clusters of small-sized adipocytes containing multiple lipid vesicles was abundantly present in iWAT of NAM HD mice compared with untreated mice, suggesting a shift into a brown-like phenotype. Indeed, NAM administration stimulated a brown/beige fat thermogenic gene program of this inguinal fat depot. In addition to the upregulation of iWAT *Pgc1a* and *Pgc1b* genes, relative *Ucp1* mRNA levels (10.4-fold, P<0.05) and Ucp1 protein abundance were significantly increased in NAM HD mice (:2.5-fold, P<0.05) compared with untreated mice (**Figure 5, panels B and C**).

Mitochondrial β-oxidation was significantly elevated in iWAT (NAM LD: 1.9-fold, P<0.05; NAM HD: 1.4-fold, P<0.05) of NAM-treated mice (**Figure 5, panel D**), suggesting that this tissue was metabolically more active in treated mice. Consistent with this view, the expression of genes involved in mitochondrial homeostasis, *Pgc1a*, *Ppargc1b*, *Mfn2* were either elevated (*Pgc1a*: 3-fold, P<0.05; *Pgc1b*: 2.5-fold, P<0.05; *Mfn2*: 4.3-fold, P<0.05) or marginally elevated (*Cpt1b*: 2.6-fold, P<0.07) upon NAM HD treatment (**Figure 5, panel**).

Given that NAM may be a main substrate for NAD+ synthesis, we evaluated the *in vivo* efficacy of NAM to increase NAD+ in iWAT. NAM treatment dose-dependently increased both total NAD and NAD+ content in iWAT. The NAD+/NADH ratio was higher in iWAT from HD NAM-treated mice (**Figure 5, panel E**). NAM treatment did not influence the relative mRNA levels of adipose

nicotinamide phosphoribosyltransferase (Nampt) or NAM riboside kinase (Nmrk)1, but augmented (2.9-fold, P<0.05) the expression of nicotinamide N-methyltransferase (Nnmt) (**Figure 5, panel G**). Thus, NAD+ elevations in adipose tissue could be due to an elevated bioavailability of NAM in this tissue due to an increased synthesis from NAD. Because AMP-activated protein kinase (AMPK) cascade is a critical regulator of brown and beige adipose tissue [30, 31], we examined the protein abundance of phosphorylated (active) AMPK in iWAT. Adipose content of phosphorylated AMPK was indeed increased (2.2-fold, P<0.05) in iWAT of NAM HD mice (**Figure 5, panel H**). Interestingly, the adipose content of AMP was significantly augmented (3.2-fold, P<0.05) in NAM HD mice compared with untreated mice (**Figure 5, panel I (CAMBIAR ORDEN DE LAS LETRAS DE PANELES EN LA FIGURA)**), consistently with its known allosteric regulation on AMPK. Phosphorylated AMPK was correlated to Ucp-1 (Pearson r = 0.59, P<0.05) (**Figure 5, panel K**).

4. Discussion

The results presented provide proof of the concept that NAM can prevent a diet-induced obesity and related complications. NAM administration very notably prevented both body weight and adiposity gain after a HFD. The mechanisms implicated involve increases in whole-body energy expenditure, at by stimulating WAT beiging, and eliciting the metabolic activity in iWAT, while reducing adipose inflammation. Importantly, NAM administration also ameliorated fatty liver and, modestly, glucose tolerance in treated mice.

Effect of NAM on adipose beiging

Adaptive thermogenesis is the main iBAT function, especially in rodents [32]. Our data did not reveal changes in the relative gene expression or protein abundance of Ucp1 in this tissue. Nevertheless, Ucp1 corrected by the total iBAT deposit was indeed increased in HD NAM-treated mice SEGUN EL PANEL DE LA FIG 4 NO HAY HIERPTROFIA DEL TEJIDO. Histological examination showed that brown adipocytes from iBAT of NAM HD mice were much less loaded with lipids than the untreated mice, suggesting that this tissue would be more active in treated mice. Also, mitochondria in iBAT might be more somewhat more metabolically active in NAM HD treated mice than in untreated mice, as revealed by the enhanced β-oxidation of fatty acids, but this does not seem to require increased gene expression of mitochondrial markers of lipolysis or biogenesis.

NAM administration prevented HFD-induced iWAT hypertrophy, as revealed by the reduced cross-sectional size of white adipocytes, and induced beiging, as shown by an increased abundance of multilocular adipocyte clusters embedded in iWAT, with an a concomitant upregulation of Ucp1, a known marker of BAT adipocytes [33]. Supporting to this finding, the relative mRNA levels of some molecular targets commonly involved in the WAT beiging (i.e., *Pgc1a*, *Pparg*) [34] were significantly upregulated or showed a trend to be induced in the inguinal fat depot of NAM HD mice. It is of note, that increased Pgc1a also leads to increased oxidative metabolism in WAT protecting from HFD-induced obesity in mice [35, 36]. Consistently, our data revealed enhanced WAT mitochondrial β-oxidation of fatty acids in NAM HD-treated mice.

Comentat [LJ1]: Josep Villena suggereix citar papers que relacionin beiging i BAT activation en reduir el pes corporal i adipositat.

NAD+ plays a key role in cellular energy metabolism, as it can be reduced to NADH, which provides reducing equivalents to the mitochondrial electron transport chain to fuel oxidative phosphorylation [7]. Additionally, NAD+ also acts

as a coenzyme for a wide range of enzymes, such as the sirtuins, particularly Sirt1, involved in the regulation of energy metabolism [7]. Significantly, AMPK is expressed in many tissues, including WAT [37]. Phosphorylated (at Thr172) levels of the α1 isoform of AMPK [37] were increased in iWAT of HD NAM treated mice. Activated AMPK also leads to enhanced oxidation of fatty acids by enhancing mitochondrial functions [38]. Consistently, mitochondrial fatty acid oxidation in WAT of NAM HD mice in correlated with a dose-dependent increase of NAD+ in this tissue. In relation to the potential action of Sirt1 in explaining AMPK activation, it is worth to note that LKB1, which phosphorylates AMPK, is also a target of Sirt1 [39].

Accumulating evidence suggests that the thermogenic activities of iBAT are activated by AMPK [40]. Indeed, cold-induced BAT activation results in the phosphorylation of AMPK to begin to increase thermogenesis. AMPK also upregulates Ucp1 and induces beiging in WAT [41] and, conceivably, could enhance iWAT beiging in NAM HD mice. I. Our data shows that the relative protein abundance of Ucp-1 was directly related to that of p-AMPK, thereby suggesting that thermogenesis could be rather induced by Ucp-1-dependent mechanisms in our treated mice.

AMPK activation may be driven by Sirt1-dependent and independent mechanisms may be involved in AMPK activation in vivo [43]. Intriguingly, adipose tissue NAD+ elevations were accompanied by a rise of adipose tissue content of AMP. The increase of AMP in WAT of treated mice is unknown, but it could be at least partly explained by different mechanisms. First, NAD+, which may be a main intracellular source of adenosine [44],[45], can conceivably increase endogenous production of adenosine, and hence contribute to the

intracellular AMP pool [46] in this tissue. Additionally, adenosine may be also formed by the hydrolysis of S-adenosylhomocysteine (SAH), which could be augmented by Nnmt. Nnmt catalyzes the transfer of a methyl group of S-adenosylmethionine (SAM) to NAM and produces SAH and me-NAM [47, 48]. In this regard, plasma concentrations of me-NAM and mRNA levels of Nnmt were upregulated in WAT. Although Nnmt activity was notdetermined , previous reports indicate that Nnmt function parallel mRNA levels in adipose tissue [11]. ADP-ribosyl produced by the cluster of differentiation (CD)38, which also uses NAD+ as a substrate [49], can subsequently be degraded to AMP [50, 51].

The effect of NAM in augmenting the NAD+ content of WAT differed from that observed in independent studies using other NAD+ intermediates, NMN and NR [10, 12]. Although we do not know the basis of such differences, they could be lattributed to the type of NAD+ precursor used and dose-related issues. Importantly, in these other studies, NAD+ precursors did not induce beiging in WAT of treated mice [10, 12]))

NAM induces an anti-inflammatory adipose phenotype

Inflammatory signalling has only recently been recognized as playing a role in adipose beiging [52], even though it is a well-known, relevant component of the adipose alterations in obesity [53]. IIt is noteworthy that chronic exposure to β-adrenergic agonists not only induces adipose beiging [54-56], but also inhibit the production of proinflammatory cytokines, such as Tnfa, and stimulate the production of anti-inflammatory cytokines, such as IL-10 [57]. The proliferation of (M2) activated macrophages is also a key feature related to WAT beiging [52, 58]. In agreement with this view, it would be feasible that the induction of beiging by NAM in iWAT could be related with the well-known anti-inflammatory NAM

effects [59, 60]. Indeed, the gene expression of *Il10*, an anti-inflammatory target and a key molecular marker of M2 macrophages in mice [61]e. Also, the relative mRNA levels of *Cd68*, a marker of macrophage infiltration, was downregulated in WAT from NAM HD mice. Consistently, the number of CLS, which commonly accumulate in fat depots from obese mice [62], were also diminished in WAT from NAM HD mice.

Several evidence support a role for adiponectin in alleviating chronic adipose tissue inflammation [63, 64]. Indeed, adiponectin induces the production of the anti-inflammatory IL-10, display M2 polarization under the influence of adiponectin. and reduces obesity-induced macrophage infiltration and inflammation [71]. Additionally, macrophages [68] In agreement with a previous report [14],.Plasma concentrations of adiponectin were elevated in response to NAM manipulation in NAM HD mice. It would be thus, attractive to suggest that plasma NAM-mediated elevations of this adipokine might contribute to the polarization of macrophages towards an M2 (i.e. anti-inflammatory) phenotype and hence elicit adaptive thermogenesis in WAT of NAM HD mice [58] [65-68] . Supporting the potential favorable impact of adiponectin in thermogenesis, AMPK also appears activated by adiponectin [72].

Our NAM HD mice also displayed a modest improvement of glucose tolerance compared with untreated mice. Adiponectin is also a widely recognized insulin signaling sensitizer [73]. Our data are consistent with a previous report [14], whereby NAM-mediated improved glucose tolerance and increased circulating levels of adiponectin in treated obese rats. Intriguingly, in this case [14], NAM treatment did not induce reduction in body weight. A possible explanation of this finding could be the differences in the experimental model and design used.

Taken together, our data, i.e., NAD+ elevations, enhanced energy expenditure as a result of increased beiging of iWAT and oxidative capacity of iBAT support combined mechanisms to explain adipose gain prevention in NAM HD mice. Reduced WAT accumulation together with tissue and systemic alleviated inflammation as a result of NAM treatment may also contribute to improve glucose homeostasis.

Limitations of the study

Supplemented water was replaced weekly. Although we did not confirmed whether NAM remained intact in drinking water at room temperature for a 7-day period, plasma concentration of NAM exhibited a dose-dependent elevation in treated mice.

Plasma concentration of NAM was not measured at the beginning of the study; however, genetically identical mice in the untreated group of DIO mice had significantly lower plasma concentration of NAM than NAM-treated mice. Although NAM content was not determined in adipose tissue, our data strongly supports the notion that NAM administration favorably influenced NAD+ biosynthesis in iWAT.

Core body temperature was not directly evaluated in this work, but iBAT histology, and protein abundance of Ucp1 suggested enhanced metabolic activity and adaptive thermogenesis.

This work provides quantifiable evidence that NAM may exerts beneficial effects on weight gain prevention. However, the contribution of other tissues also important in metabolic homeostasis, such as skeletal muscle, were not evaluated

[10, 21]. In a preliminary analysis, we found increased evaluated muscle fatty acid β-oxidation and, also there are previous studies on NAM effects in this tissue.

Conclusion

Dietary supplementation with NAM to mice prevented body weight gain and adiposity by boosting energy expenditure, with this being mainly attributed to enhanced energy demand and beiging in WAT. Interestingly, this effect was accompanied by an amelioration of WAT inflammation and fatty liver.

References

[1] A. Doria, M.E. Patti, C.R. Kahn, The emerging genetic architecture of type 2 diabetes, Cell Metab, 8 (2008) 186-200.
[2] E.A. Finkelstein, O.A. Khavjou, H. Thompson, J.G. Trogdon, L. Pan, B. Sherry, W. Dietz, Obesity and severe obesity forecasts through 2030, Am J Prev Med, 42 (2012) 563-570.
[3] S.M. Grundy, Metabolic complications of obesity, Endocrine, 13 (2000) 155-165.
[4] A. Vegiopoulos, M. Rohm, S. Herzig, Adipose tissue: between the extremes, EMBO J, 36 (2017) 1999-2017.
[5] K. Makki, P. Froguel, I. Wolowczuk, Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines, ISRN Inflamm, 2013 (2013) 139239.
[6] M. Keuper, S. Sachs, E. Walheim, L. Berti, B. Raedle, D. Tews, P. Fischer-Posovszky, M. Wabitsch, M. Hrabe de Angelis, G. Kastenmuller, M.H. Tschop, M. Jastroch, H. Staiger, S.M. Hofmann, Activated macrophages control human adipocyte mitochondrial bioenergetics via secreted factors, Mol Metab, 6 (2017) 1226-1239.
[7] C. Canto, K.J. Menzies, J. Auwerx, NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus, Cell Metab, 22 (2015) 31-53.
[8] L. Rajman, K. Chwalek, D.A. Sinclair, Therapeutic Potential of NAD-Boosting Molecules: The In Vivo Evidence, Cell Metab, 27 (2018) 529-547.
[9] P. Bai, C. Canto, H. Oudart, A. Brunyanszki, Y. Cen, C. Thomas, H. Yamamoto, A. Huber, B. Kiss, R.H. Houtkooper, K. Schoonjans, V. Schreiber, A.A. Sauve, J. Menissier-de Murcia, J. Auwerx, PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation, Cell Metab, 13 (2011) 461-468.
[10] C. Canto, R.H. Houtkooper, E. Pirinen, D.Y. Youn, M.H. Oosterveer, Y. Cen, P.J. Fernandez-Marcos, H. Yamamoto, P.A. Andreux, P. Cettour-Rose, K. Gademann, C. Rinsch, K. Schoonjans, A.A. Sauve, J. Auwerx, The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity, Cell Metab, 15 (2012) 838-847.
[11] D. Kraus, Q. Yang, D. Kong, A.S. Banks, L. Zhang, J.T. Rodgers, E. Pirinen, T.C. Pulinilkunnil, F. Gong, Y.C. Wang, Y. Cen, A.A. Sauve, J.M. Asara, O.D. Peroni, B.P. Monia, S. Bhanot, L. Alhonen, P. Puigserver, B.B. Kahn, Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity, Nature, 508 (2014) 258-262.

[12] J. Yoshino, K.F. Mills, M.J. Yoon, S. Imai, Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice, Cell Metab, 14 (2011) 528-536.
[13] E. Pirinen, C. Canto, Y.S. Jo, L. Morato, H. Zhang, K.J. Menzies, E.G. Williams, L. Mouchiroud, N. Moullan, C. Hagberg, W. Li, S. Timmers, R. Imhof, J. Verbeek, A. Pujol, B. van Loon, C. Viscomi, M. Zeviani, P. Schrauwen, A.A. Sauve, K. Schoonjans, J. Auwerx, Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle, Cell Metab, 19 (2014) 1034-1041.
[14] S.J. Yang, J.M. Choi, L. Kim, S.E. Park, E.J. Rhee, W.Y. Lee, K.W. Oh, S.W. Park, C.Y. Park, Nicotinamide improves glucose metabolism and affects the hepatic NAD-sirtuin pathway in a rodent model of obesity and type 2 diabetes, J Nutr Biochem, 25 (2014) 66-72.
[15] J. Yoshino, J.A. Baur, S.I. Imai, NAD(+) Intermediates: The Biology and Therapeutic Potential of NMN and NR, Cell Metab, 27 (2018) 513-528.
[16] C. Canto, Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity, Nature, 458 (2009) 1056-1060.
[17] C. Canto, L.Q. Jiang, A.S. Deshmukh, C. Mataki, A. Coste, M. Lagouge, J.R. Zierath, J. Auwerx, Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle, Cell Metab, 11 (2010) 213-219.
[18] S.R. Costford, S. Bajpeyi, M. Pasarica, D.C. Albarado, S.C. Thomas, H. Xie, T.S. Church, S.A. Jubrias, K.E. Conley, S.R. Smith, Skeletal muscle NAMPT is induced by exercise in humans, Am J Physiol Endocrinol Metab, 298 (2010) E117-126.
[19] S.R. Costford, B. Brouwers, M.E. Hopf, L.M. Sparks, M. Dispagna, A.P. Gomes, H.H. Cornnell, C. Petucci, P. Phelan, H. Xie, F. Yi, G.A. Walter, T.F. Osborne, D.A. Sinclair, R.L. Mynatt, J.E. Ayala, S.J. Gardell, S.R. Smith, Skeletal muscle overexpression of nicotinamide phosphoribosyl transferase in mice coupled with voluntary exercise augments exercise endurance, Mol Metab, 7 (2018) 1-11.
[20] D. Chen, J. Bruno, E. Easlon, S.J. Lin, H.L. Cheng, F.W. Alt, L. Guarente, Tissue-specific regulation of SIRT1 by calorie restriction, Genes Dev, 22 (2008) 1753-1757.
[21] K.F. Mills, S. Yoshida, L.R. Stein, A. Grozio, S. Kubota, Y. Sasaki, P. Redpath, M.E. Migaud, R.S. Apte, K. Uchida, J. Yoshino, S.I. Imai, Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice, Cell Metab, 24 (2016) 795-806.
[22] R.B. Elliott, C.C. Pilcher, A. Stewart, D. Fergusson, M.A. McGregor, The use of nicotinamide in the prevention of type 1 diabetes, Ann N Y Acad Sci, 696 (1993) 333-341.
[23] S.J. Mitchell, M. Bernier, M.A. Aon, S. Cortassa, E.Y. Kim, E.F. Fang, H.H. Palacios, A. Ali, I. Navas-Enamorado, A. Di Francesco, T.A. Kaiser, T.B. Waltz, N. Zhang, J.L. Ellis, P.J. Elliott, D.W. Frederick, V.A. Bohr, M.S. Schmidt, C. Brenner, D.A. Sinclair, A.A. Sauve, J.A. Baur, R. de Cabo, Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice, Cell Metab, 27 (2018) 667-676 e664.
[24] M. Komatsu, T. Kanda, H. Urai, A. Kurokochi, R. Kitahama, S. Shigaki, T. Ono, H. Yukioka, K. Hasegawa, H. Tokuyama, H. Kawabe, S. Wakino, H. Itoh, NNMT activation can contribute to the development of fatty liver disease by modulating the NAD (+) metabolism, Sci Rep, 8 (2018) 8637.
[25] K.A. Mendez-Lara, D. Santos, N. Farre, S. Ruiz-Nogales, S. Leanez, J.L. Sanchez-Quesada, E. Zapico, E. Lerma, J.C. Escola-Gil, F. Blanco-Vaca, J.M. Martin-Campos, J. Julve, O. Pol, Administration of CORM-2 inhibits diabetic neuropathy but does not reduce dyslipidemia in diabetic mice, PLoS One, 13 (2018) e0204841.

[26] P.B. Lazarow, Assay of peroxisomal beta-oxidation of fatty acids, Methods Enzymol, 72 (1981) 315-319.
[27] J. Wu, P. Bostrom, L.M. Sparks, L. Ye, J.H. Choi, A.H. Giang, M. Khandekar, K.A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W.D. van Marken Lichtenbelt, J. Hoeks, S. Enerback, P. Schrauwen, B.M. Spiegelman, Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human, Cell, 150 (2012) 366-376.
[28] G. Ferrannini, M. Namwanje, B. Fang, M. Damle, D. Li, Q. Liu, M.A. Lazar, L. Qiang, Genetic backgrounds determine brown remodeling of white fat in rodents, Mol Metab, 5 (2016) 948-958.
[29] S. Collins, K.W. Daniel, A.E. Petro, R.S. Surwit, Strain-specific response to beta 3-adrenergic receptor agonist treatment of diet-induced obesity in mice, Endocrinology, 138 (1997) 405-413.
[30] E.M. Desjardins, G.R. Steinberg, Emerging Role of AMPK in Brown and Beige Adipose Tissue (BAT): Implications for Obesity, Insulin Resistance, and Type 2 Diabetes, Curr Diab Rep, 18 (2018) 80.
[31] S. Wang, X. Liang, Q. Yang, X. Fu, C.J. Rogers, M. Zhu, B.D. Rodgers, Q. Jiang, M.V. Dodson, M. Du, Resveratrol induces brown-like adipocyte formation in white fat through activation of AMP-activated protein kinase (AMPK) alpha1, Int J Obes (Lond), 39 (2015) 967-976.
[32] L.P. Kozak, R.A. Koza, R. Anunciado-Koza, Brown fat thermogenesis and body weight regulation in mice: relevance to humans, Int J Obes (Lond), 34 Suppl 1 (2010) S23-27.
[33] B. Cannon, J. Nedergaard, Brown adipose tissue: function and physiological significance, Physiol Rev, 84 (2004) 277-359.
[34] F. Villarroya, M. Peyrou, M. Giralt, Transcriptional regulation of the uncoupling protein-1 gene, Biochimie, 134 (2017) 86-92.
[35] L. Ye, S. Kleiner, J. Wu, R. Sah, R.K. Gupta, A.S. Banks, P. Cohen, M.J. Khandekar, P. Bostrom, R.J. Mepani, D. Laznik, T.M. Kamenecka, X. Song, W. Liedtke, V.K. Mootha, P. Puigserver, P.R. Griffin, D.E. Clapham, B.M. Spiegelman, TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis, Cell, 151 (2012) 96-110.
[36] M. Yan, E. Audet-Walsh, S. Manteghi, C.R. Dufour, B. Walker, M. Baba, J. St-Pierre, V. Giguere, A. Pause, Chronic AMPK activation via loss of FLCN induces functional beige adipose tissue through PGC-1alpha/ERRalpha, Genes Dev, 30 (2016) 1034-1046.
[37] J.H. Um, J.S. Pendergast, D.A. Springer, M. Foretz, B. Viollet, A. Brown, M.K. Kim, S. Yamazaki, J.H. Chung, AMPK regulates circadian rhythms in a tissue- and isoform-specific manner, PLoS One, 6 (2011) e18450.
[38] G.R. Steinberg, B.E. Kemp, AMPK in Health and Disease, Physiol Rev, 89 (2009) 1025-1078.
[39] F. Lan, J.M. Cacicedo, N. Ruderman, Y. Ido, SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation, J Biol Chem, 283 (2008) 27628-27635.
[40] A.D. van Dam, S. Kooijman, M. Schilperoort, P.C. Rensen, M.R. Boon, Regulation of brown fat by AMP-activated protein kinase, Trends Mol Med, 21 (2015) 571-579.
[41] L. Wu, L. Zhang, B. Li, H. Jiang, Y. Duan, Z. Xie, L. Shuai, J. Li, J. Li, AMP-Activated Protein Kinase (AMPK) Regulates Energy Metabolism through Modulating Thermogenesis in Adipose Tissue, Front Physiol, 9 (2018) 122.
[42] A.E. Pollard, L. Martins, P.J. Muckett, S. Khadayate, A. Bornot, M. Clausen, T. Admyre, M. Bjursell, R. Fiadeiro, L. Wilson, C. Whilding, V.N. Kotiadis, M.R.

Duchen, D. Sutton, L. Penfold, A. Sardini, Y.M. Bohlooly, D.M. Smith, J.A. Read, M.A. Snowden, A. Woods, D. Carling, AMPK activation protects against diet induced obesity through Ucp1-independent thermogenesis in subcutaneous white adipose tissue, Nat Metab, 1 (2019) 340-349.
[43] N.L. Price, A.P. Gomes, A.J. Ling, F.V. Duarte, A. Martin-Montalvo, B.J. North, B. Agarwal, L. Ye, G. Ramadori, J.S. Teodoro, B.P. Hubbard, A.T. Varela, J.G. Davis, B. Varamini, A. Hafner, R. Moaddel, A.P. Rolo, R. Coppari, C.M. Palmeira, R. de Cabo, J.A. Baur, D.A. Sinclair, SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function, Cell Metab, 15 (2012) 675-690.
[44] J. Linden, F. Koch-Nolte, G. Dahl, Purine Release, Metabolism, and Signaling in the Inflammatory Response, Annu Rev Immunol, 37 (2019) 325-347.
[45] J. Zhang, C. Wang, H. Shi, D. Wu, W. Ying, Extracellular Degradation Into Adenosine and the Activities of Adenosine Kinase and AMPK Mediate Extracellular NAD(+)-Produced Increases in the Adenylate Pool of BV2 Microglia Under Basal Conditions, Front Cell Neurosci, 12 (2018) 343.
[46] D. Boison, Adenosine kinase: exploitation for therapeutic gain, Pharmacol Rev, 65 (2013) 906-943.
[47] S. Aksoy, B.F. Brandriff, A. Ward, P.F. Little, R.M. Weinshilboum, Human nicotinamide N-methyltransferase gene: molecular cloning, structural characterization and chromosomal localization, Genomics, 29 (1995) 555-561.
[48] J. Rini, C. Szumlanski, R. Guerciolini, R.M. Weinshilboum, Human liver nicotinamide N-methyltransferase: ion-pairing radiochemical assay, biochemical properties and individual variation, Clin Chim Acta, 186 (1990) 359-374.
[49] E.N. Chini, CD38 as a regulator of cellular NAD: a novel potential pharmacological target for metabolic conditions, Curr Pharm Des, 15 (2009) 57-63.
[50] A.L. Horenstein, A. Chillemi, V. Quarona, A. Zito, I. Roato, F. Morandi, D. Marimpietri, M. Bolzoni, D. Toscani, R.J. Oldham, M. Cuccioloni, A.K. Sasser, V. Pistoia, N. Giuliani, F. Malavasi, NAD(+)-Metabolizing Ectoenzymes in Remodeling Tumor-Host Interactions: The Human Myeloma Model, Cells, 4 (2015) 520-537.
[51] A.L. Horenstein, V. Quarona, D. Toscani, F. Costa, A. Chillemi, V. Pistoia, N. Giuliani, F. Malavasi, Adenosine Generated in the Bone Marrow Niche Through a CD38-Mediated Pathway Correlates with Progression of Human Myeloma, Mol Med, 22 (2016) 694-704.
[52] B. Chaurasia, V.A. Kaddai, G.I. Lancaster, D.C. Henstridge, S. Sriram, D.L. Galam, V. Gopalan, K.N. Prakash, S.S. Velan, S. Bulchand, T.J. Tsong, M. Wang, M.M. Siddique, G. Yuguang, K. Sigmundsson, N.A. Mellet, J.M. Weir, P.J. Meikle, M.Y.M.S. Bin, A. Shabbir, J.A. Shayman, Y. Hirabayashi, S.T. Shiow, S. Sugii, S.A. Summers, Adipocyte Ceramides Regulate Subcutaneous Adipose Browning, Inflammation, and Metabolism, Cell Metab, 24 (2016) 820-834.
[53] D.M. Mosser, J.P. Edwards, Exploring the full spectrum of macrophage activation, Nat Rev Immunol, 8 (2008) 958-969.
[54] J. Himms-Hagen, J. Cui, E. Danforth, Jr., D.J. Taatjes, S.S. Lang, B.L. Waters, T.H. Claus, Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats, Am J Physiol, 266 (1994) R1371-1382.
[55] C. Guerra, R.A. Koza, H. Yamashita, K. Walsh, L.P. Kozak, Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity, J Clin Invest, 102 (1998) 412-420.

[56] B. Cousin, S. Cinti, M. Morroni, S. Raimbault, D. Ricquier, L. Penicaud, L. Casteilla, Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization, J Cell Sci, 103 (Pt 4) (1992) 931-942.
[57] I.J. Elenkov, G.P. Chrousos, Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity, Ann N Y Acad Sci, 966 (2002) 290-303.
[58] X. Hui, P. Gu, J. Zhang, T. Nie, Y. Pan, D. Wu, T. Feng, C. Zhong, Y. Wang, K.S. Lam, A. Xu, Adiponectin Enhances Cold-Induced Browning of Subcutaneous Adipose Tissue via Promoting M2 Macrophage Proliferation, Cell Metab, 22 (2015) 279-290.
[59] R. Biedron, M. Ciszek, M. Tokarczyk, M. Bobek, M. Kurnyta, E.M. Slominska, R.T. Smolenski, J. Marcinkiewicz, 1-Methylnicotinamide and nicotinamide: two related anti-inflammatory agents that differentially affect the functions of activated macrophages, Arch Immunol Ther Exp (Warsz), 56 (2008) 127-134.
[60] R. Weiss, E. Schilling, A. Grahnert, V. Kolling, J. Dorow, U. Ceglarek, U. Sack, S. Hauschildt, Nicotinamide: a vitamin able to shift macrophage differentiation toward macrophages with restricted inflammatory features, Innate Immun, 21 (2015) 813-826.
[61] D.M. Mosser, The many faces of macrophage activation, J Leukoc Biol, 73 (2003) 209-212.
[62] S. Cinti, G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, S. Wang, M. Fortier, A.S. Greenberg, M.S. Obin, Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans, J Lipid Res, 46 (2005) 2347-2355.
[63] N. Ouchi, K. Walsh, Adiponectin as an anti-inflammatory factor, Clin Chim Acta, 380 (2007) 24-30.
[64] A.T. Turer, P.E. Scherer, Adiponectin: mechanistic insights and clinical implications, Diabetologia, 55 (2012) 2319-2326.
[65] K. Ohashi, J.L. Parker, N. Ouchi, A. Higuchi, J.A. Vita, N. Gokce, A.A. Pedersen, C. Kalthoff, S. Tullin, A. Sams, R. Summer, K. Walsh, Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype, J Biol Chem, 285 (2010) 6153-6160.
[66] A.M. Wolf, D. Wolf, H. Rumpold, B. Enrich, H. Tilg, Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes, Biochem Biophys Res Commun, 323 (2004) 630-635.
[67] M. Kumada, S. Kihara, N. Ouchi, H. Kobayashi, Y. Okamoto, K. Ohashi, K. Maeda, H. Nagaretani, K. Kishida, N. Maeda, A. Nagasawa, T. Funahashi, Y. Matsuzawa, Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages, Circulation, 109 (2004) 2046-2049.
[68] P. Mandal, B.T. Pratt, M. Barnes, M.R. McMullen, L.E. Nagy, Molecular mechanism for adiponectin-dependent M2 macrophage polarization: link between the metabolic and innate immune activity of full-length adiponectin, J Biol Chem, 286 (2011) 13460-13469.
[69] T. Yokota, K. Oritani, I. Takahashi, J. Ishikawa, A. Matsuyama, N. Ouchi, S. Kihara, T. Funahashi, A.J. Tenner, Y. Tomiyama, Y. Matsuzawa, Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages, Blood, 96 (2000) 1723-1732.
[70] M.C. Wulster-Radcliffe, K.M. Ajuwon, J. Wang, J.A. Christian, M.E. Spurlock, Adiponectin differentially regulates cytokines in porcine macrophages, Biochem Biophys Res Commun, 316 (2004) 924-929.

[71] J.Y. Kim, E. van de Wall, M. Laplante, A. Azzara, M.E. Trujillo, S.M. Hofmann, T. Schraw, J.L. Durand, H. Li, G. Li, L.A. Jelicks, M.F. Mehler, D.Y. Hui, Y. Deshaies, G.I. Shulman, G.J. Schwartz, P.E. Scherer, Obesity-associated improvements in metabolic profile through expansion of adipose tissue, J Clin Invest, 117 (2007) 2621-2637.
[72] S. Cardaci, G. Filomeni, M.R. Ciriolo, Redox implications of AMPK-mediated signal transduction beyond energetic clues, J Cell Sci, 125 (2012) 2115-2125.
[73] H. Ruan, L.Q. Dong, Adiponectin signaling and function in insulin target tissues, J Mol Cell Biol, 8 (2016) 101-109.

Table 1. Effect of NAM administration on gross parameters and plasma

	Untreated	NAM LD	NAM HD	P
Gross parameters				
Body weight [g]	35.04 (1.47)	32.86 (4.06)†	28.11 (1.28)*	<0.05
Final weight gain [g]	11.40 (2.17)	7.32 (0.75)*†	3.48 (1.01)*	<0.05
Fat Pad [g]	4.55 (1.55)	2.55 (0.50)*†	1.31 (0.19)*	<0.05
Lean Weight [g]	31.17 (1.26)	28.54 (1.36)*	27.11 (1.14)*	<0.05
Liver weight [g]	1.21 (0.26)	1.08 (0.28)	1.06 (0.22)*	<0.05
Liver-to-body weight ratio	0.03 (0.00)	0.03 (0.00)	0.04 (0.00)	<0.05
Diet intake [g/day]	2.74 (0.63)	2.92 (0.22)	2.99 (0.56)	0.34
Water intake [g/day]	4.95 (1.58)	4.17 (0.77)	4.39 (1.26)	0.31
Calculated dose [g/kg/day]		0.32 (0.08)	1.40 (0.26)	
Biochemical parameters				
Glucose [mM]	13.18 (1.85)	12.92 (2.16)	15.19 (1.47)	0.13
Insulin [ng/mL]	0.48 (0.07)	0.39 (0.05)	0.39 (0.07)	0.63
Total cholesterol [mM]	3.97 (1.07)	3.71 (0.53)	3.88 (0.10)	0.30

Triglycerides [mM]	0.83 (0.31)	1.01 (0.40)	1.44 (0.57)	0.16
FFA [mM]	0.85 (0.11)	0.67 (0.05)*	0.54 (0.10)*	<0.05
Adiponectin [ng/mL]	75.96 (11.13)	n. d.	105.40 (15.80)	<0.05
Leptin [ng/mL]	30,809 (16,913)	n. d.	6,540 (4,311)	<0.05

biochemistry.

Results are expressed as the means (standard deviation) (n = 8 mice per group). All analyses were made at three months of age. At the age of two months the mice were challenged to high-fat diet and NAM manipulation for three months. Food intake was measured at the end of the study as described in the Materials and Methods section. Differences between the mean values were assessed by the nonparametric a Kruskal Wallis followed by Dunn's posttest or ANOVA followed a Newman-Keuls posttest, as appropriate; differences were considered significant when $P<0.05$. Specifically, # $P<0.05$ vs. non-obese group, * $P<0.05$ vs. untreated group; or † $P<0.05$ vs. NAM LD-treated mice. Abbreviations used: NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice, FFA, free fatty acids; HDL, high-density lipoprotein; n. d., not determined.

Supplementary Table 1. Transitions used in QqQ shown by different metabolites.

Metabolite	RT (min)	1st Transition (CE (V))	2nd Transition (CE (V))
N-Me-Nicotinamide	0.76	137 → 80 (24)	137 → 108 (16)
d_4-Nicotinamide (IS)	0.91	127 → 84 (24)	127 → 81 (28)
Nicotinamide	0.93	123 → 80 (20)	123 → 53 (32)

	DIO			
	untreated	NAM LD	NAM HD	P
NAM [µM]	17.80 (30.32)	177.50 (84.94)*†	568.40 (51.45)*	<0.05
Me-NAM [µM]	2.2e-004 (1.6e-004)	2.5e-004 (9.2e-005)†	7.1e-0.004 (2.3e-004)*	<0.05
Creatinine [mM]	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.23
BUN [µM]	7.58 (2.00)	6.94 (1.60)	8.69 (1.93)	0.23
AST [U/L]	152.8 (61.04)	173.1 (66.15)	124.6 (58.06)	0.56
ALT [U/L]	59.18 (46.07)	39.17 (17.77)	21.98 (6.49)	0.27

Urine [mM]	Creatinine	3.33 (0.54)	3.14 (0.86)	3.35 (1.83)	0.85

Supplementary Table 2. Effect of NAM on hepatic and renal parameters in DIO mice. Results are expressed as the means (standard deviation) (n = 8 mice per group). At the age of two months the mice were challenged to high-fat diet and NAM manipulation for three months. Plasma concentration of NAM and me-NAM were given in relative units (n = 5-6 mice per group). Differences between the mean values were assessed by the nonparametric a Kruskal Wallis followed by Dunn's posttest; differences were considered significant when $P<0.05$. Specifically, *$P<0.05$ vs. untreated group. Abbreviations used: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; NAM LD, low-dose, NAM-treated mice; NAM HD, high-dose, NAM-treated mice.