Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 36 (2016) 31–41

Journal of Nutritional Biochemistry

Long-term treatment with nicotinamide induces glucose intolerance and skeletal muscle lipotoxicity in normal chow-fed mice: compared to diet-induced obesity☆

Zhengtang Qi*, Jie Xia, Xiangli Xue, Qiang He, Liu Ji, Shuzhe Ding*

The Key Laboratory of Adolescent Health Assessment and Exercise Intervention (East China Normal University), Ministry of Education, Shanghai 200241, China
College of Physical Education and Health, East China Normal University, Shanghai 200241, China

Received 22 March 2016; received in revised form 6 May 2016; accepted 5 July 2016

Abstract

Nicotinamide (NAM), or vitamin B3, is an essential coenzyme for ATP synthesis and an inhibitor of sirtuin 1. Recently, conflicting results were reported regarding the treatment of NAM in type 2 diabetes and obesity. The aim of this study was to determine whether and how long-term treatment with NAM at lower dose would affect insulin sensitivity in mice fed chow diet. We treated mice with NAM (100 mg/kg/day) and normal chow for 8 weeks. Strikingly, NAM induced glucose intolerance and skeletal muscle lipid accumulation in nonobese mice. NAM impaired mitochondrial respiration capacity and energy production in skeletal muscle, in combination with increased expression of the mediators for mitophagy (p62, PINK1, PARK2 and NIX) and autophagy (FOXO3, Bnip3, CTSL, Beclin1 and LC-3b). Next, we treated mice with high-fat diet (HFD) and resveratrol (RSV; 100 mg/kg/day) for 8 weeks. RSV protected against HFD-induced insulin resistance and obesity. HFD increased skeletal muscle lipid content as well as NAM, but this increase was attenuated by RSV. In contrast to NAM, HFD enhanced fatty acid oxidative capacity. Muscle transcript levels of genes for mitophagy and autophagy were largely suppressed by HFD, whereas RSV did not rescue these effects. These differences suggest that skeletal muscle autophagy may represent adaptive response to NAM-induced lipotoxicity, whereas reduced autophagy in skeletal muscle may promote HFD-induced lipotoxicity. Our results demonstrate that chronic NAM supplementation in healthy individuals, although at lower dose than previously reported, is still detrimental to glucose homeostasis and skeletal muscle lipid metabolism.
© 2016 Elsevier Inc. All rights reserved.

Keywords: Nicotinamide (NAM); Sirtuin 1; Insulin resistance; Lipid accumulation; Skeletal muscle; Mitochondrial; Mitophagy

1. Introduction

Sirtuin 1 (SIRT1), a NAD-dependent histone deacetylase, plays a positive role in metabolic pathway through its deacetylase activity for a number of substrates involved in glucose or lipid metabolism [1,2]. As a known activator of SIRT1, resveratrol (RSV) improves mitochondrial function and protects against diet-induced obesity and insulin resistance [3]. SIRT1 is required for the beneficial effects of RSV on mitochondrial function [4]. Nicotinamide (NAM), a form of vitamin B3 and a NAD+ precursor, is a potent inhibitor of SIRT1 [5]. NAM physically inhibits SIRT1 deacetylation activity and increases the levels of acetylated histone and p53 [6]. Previously, NAM was observed to have preventive and therapeutic effects on type 1 diabetes and protected β-cell against the toxic effects of alloxan and streptozocin [7–9]. Evidence from animal and human studies suggested that NAM can be used as a dietary micronutrient to protect against the development of insulin-dependent diabetes mellitus (IDDM). Recent studies indicated that NAM treatment (100 mg/kg, 4 weeks) improved glucose metabolism in a rodent model of obesity and type 2 diabetes [10]. Nicotinamide riboside (NR) can be used as a natural vitamin to enhance oxidative metabolism, protect against diet-induced obesity and ameliorate metabolic and age-related disorders characterized by defective mitochondrial function [11]. These findings indicate that NAM is an antidiabetic vitamin for both type 1 and type 2 diabetes. If so, there may be a paradox in the regulation of SIRT1. In contrast to NAM as an inhibitor of SIRT1, RSV is a SIRT1 activator used for improving insulin resistance and mitochondrial function [3]. It is noteworthy that NAM is also a substrate for nicotinamide *N*-methyltransferase (NNMT), which methylates NAM to produce *N*(1)-methylnicotinamide (MNAM). Recent studies have suggested that NNMT activity and plasma MNAM levels are linked to diet-induced obesity and diabetes in rodents and humans [12–14]. Thus, the metabolic effects of NAM supplementation are very likely not only the result of the inhibition of SIRT1 activity. Another paradox is due to

Abbreviations: BSA, bovine serum albumin; DAG, diacylglycerol; FA, fatty acids; FFA, free fatty acids; GTT, glucose tolerance tests; HFD, high-fat diet; IDDM, insulin-dependent diabetes mellitus; IPGTT, intraperitoneal glucose tolerance tests; ITT, insulin tolerance tests; MNAM, *N*(1)-methylnicotinamide; MRI, magnetic resonance imaging; mtDNA, mitochondrial DNA; NAM, nicotinamide; NNMT, nicotinamide *N*-methyltransferase; NR, nicotinamide riboside; RSV, resveratrol; SIRT1, sirtuin 1; T2DM, type 2 diabetes mellitus.

☆ Disclosure Statement: The authors have nothing to disclose.

* Corresponding authors. College of Physical Education and Health, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China. Tel./fax: +86-21-54345296.

E-mail addresses: qzht79@163.com (Z. Qi), szding@tyxx.ecnu.edu.cn (S. Ding).

http://dx.doi.org/10.1016/j.jnutbio.2016.07.005
0955-2863/© 2016 Elsevier Inc. All rights reserved.

the dosage of NAM treatment. Previous studies showed that NAM administration (2 g/day, 2 weeks) caused a decrease in insulin sensitivity in IDDM patients despite increased insulin secretion, suggesting that the use of NAM in IDDM may lead to insulin resistance in humans [15]. Animal study also reported that NAM supplementation (4 g/kg, 8 weeks) induced detrimental metabolic changes in young rats, as evidenced by increased hepatic DNA damage and impaired glucose tolerance and insulin sensitivity [16]. Cumulative administration of NAM (2 g/kg) promoted the development of type 2 diabetes mellitus (T2DM) [17]. Excess NAM intake leads to homocysteine-related cardiovascular disease [18]. The antidiabetic role of NAM as a vitamin and the associated underlying mechanisms need to be further understood.

Lipotoxicity has been defined as the accumulation of lipid in nonadipose tissue, leading to cellular dysfunction and death. However, triglyceride (TAG) within skeletal muscle is thought to be the best fuel for healthy muscle during exercise [19], although intramuscular TAG is an important contributor to obesity, insulin resistance and type 2 diabetes. The content of skeletal muscle lipids is known as a marker of insulin resistance, while these neutral triglycerides are not thought to be harmful [20,21]. Intramuscular lipid content was increased in obese subjects with T2DM while glycogen stores were simultaneously reduced [22], suggesting a pathway to modulate oxidative substrate selection and induce muscle insulin resistance. Surprisingly, recent findings demonstrate that modulation of oxidative substrate selection to increase muscle glucose utilization also results in muscle insulin resistance [23]. Insulin resistance did not increase proportionally to fat mass or lipid accumulation in obese and nonobese subjects with T2DM [24]. Thus, the balance between storage and utilization of TAG is critical for understanding the role of skeletal muscle lipid accumulation in insulin resistance. Mechanistically, reduced skeletal muscle mitochondrial activity results in ectopic lipid accumulation and reduced glucose tolerance in elderly adults, independent of obesity [25]. Skeletal muscle fibers differentiate into specific fiber types with distinct metabolic properties determined by their oxidative substrates. Mitochondrial quality control is mostly determined by mitochondrial biogenesis, fusion and fission and mitochondrial autophagy (*i.e.* mitophagy) [26,27]. PGC-1α is a master nuclear coactivator to mediate skeletal muscle mitochondrial biogenesis and enhance lipid-supported mitochondrial respiration [28,29]. Conversely, nuclear corepressors NCoR1 and RIP140 have been documented to antagonize the transcription activation of PGC-1α and inhibit muscle mitochondrial biogenesis and oxidative metabolism [30,31]. In addition, previous studies have investigated the importance of autophagy in metabolic regulation. Skeletal muscle autophagy is essential for muscle mass, mitochondrial function and insulin sensitivity [32]. Induction of autophagy decreased lipid accumulation and increased oxidative phosphorylation in hepatic cells. Rapamycin treatment induced autophagy and decreased hepatic TAG and glycogen content, as well as corrected hepatic lipid accumulation [33]. Endothelial autophagy is critically important for limiting lipid accumulation within the vessel wall. Induction of autophagy prevented the age-dependent decline in autophagic flux and treated atherosclerosis [34]. These findings had linked autophagy to ectopic lipid accumulation and the related diseases.

Ecological studies have shown that increased B vitamin consumption is strongly correlated with the prevalence of obesity and diabetes [35]. Although previous studies emphasized the beneficial or detrimental effects of NAM in treatment of obesity and type 2 diabetes, the understanding of how NAM modulates lipid metabolism has not been directly addressed *in vivo*. To examine this question, we sought to determine whether long-term treatment with NAM (100 mg/kg/day, 8 weeks) would affect insulin sensitivity and skeletal muscle lipid metabolism. We found that NAM induced insulin resistance and skeletal muscle lipotoxicity in nonobese mice. Compared to diet-induced obesity and insulin resistance, NAM specifically increased lipid accumulation in skeletal muscle rather than in adipose tissue and liver.

2. Materials and methods

2.1. Mice and drugs treatment

Male C57/BL6 mice were obtained from Sino-British Sippr/BK Lab Animal (Shanghai, China) and Jackson Laboratories. The animals were housed in plastic cages in a room kept at a room temperature of 23±2°C and a light–dark cycle, and mice had *ad libitum* access to water and food. Mice were fed regular chow (18% fat, 58% carbohydrate and 24% protein by calories; Harlan Teklad) or 8 weeks of high-fat diet (HFD; 55% fat, 24% carbohydrate and 21% protein by calories; Harlan Teklad). For chronic treatment with NAM and RSV, the mice received intraperitoneal injection at the dose of 100 mg/kg body weight/day for 8 weeks. The same volume of vehicle (10% DMSO in saline) was injected to the control mice. Fat and lean body mass was assessed by magnetic resonance spectroscopy [magnetic resonance imaging (MRI)]. All experiments were performed in accordance with the guidelines established by China Ministry of Health for the Care and Use of Laboratory Animals. Experimental procedures were approved by the Experimental Animal Care and Use Committee at East China Normal University (ECNU 2006-05) and the local research institution. Every effort was made to optimize comfort and to minimize the use of animals.

2.2. Glucose and insulin tolerance tests

Three days after the last drug injection, the mice were deprived of food for 16 h and then subjected to glucose tolerance tests (GTT) and insulin tolerance tests (ITT). GTT were performed as previously described [36]. Mice were injected intraperitoneally with glucose (1 g/kg), and blood was collected by tail bleed at 0, 15, 30, 45, 60, 90 and 120 min for plasma glucose measurements. For ITT, mice were injected intraperitoneally with insulin (0.75 U/kg), and blood was collected for plasma glucose measurements.

2.3. Blood analysis and tissue lipid content

Three days after the last drug injection, the mice were sacrificed to collect blood and tissues. Fasting serum glucose, triglyceride (TG) and free fatty acids (FFA) were determined by enzymatic colorimetric assays according to the manufacturer's instruction. Serum insulin was measured by ELISA (R&D Systems, USA). Tissue triglyceride was extracted as described previously [37] and measured by an enzymatic assay (Sigma-Aldrich). Diacylglycerol (DAG) and ceramides were extracted and subjected to liquid chromatography–tandem mass spectrometry analysis as described previously [37]. [NAD+]/[NADH] ratio was measured as described by So-young Jang [5]. Tissues were homogenized in the assay buffer and centrifuged for lactate oxidase assay and pyruvate oxidase assay; the ratio of pyruvate/lactate was used to calculate [NAD+]/[NADH] ratio.

2.4. Glycogen content assays

Approximately 100 mg of tissues was homogenized in 10:1 volume/weight dilution, and glycogen content was measured as described previously [38]. Briefly, homogenates were centrifuged at 5000*g* for 5 min at 4°C. The supernatants or glycogen standards were spotted onto GF/A filters. Filters were washed in ice-cold ethanol for 15 min followed by two washes in 70% ethanol at room temperature. After drying overnight, filters were placed in glass tubes and incubated with 1 ml amyloglucosidase (0.04% in 0.05 M sodium acetate) for 90 min at 37°C. Glucose present in the reaction was analyzed using a commercial kit according to the manufacturer's instructions.

2.5. Fatty acid metabolism

As described previously [39], muscle strips were equilibrated in 2 ml of pregassed Krebs–Henseleit buffer [4% bovine serum albumin (BSA), 30°C] with 1 mM palmitate and 5 mM glucose in a gentle shaking bath for 30 min. Muscle strips continued to be incubated for additional 60 min with the addition of 0.5 μCi/ml [1-^{14}C]palmitate. Exogenous palmitate oxidation and esterification were monitored by the production of $^{14}CO_2$ and incorporation of [1-^{14}C] palmitate into intramuscular lipids, respectively.

2.6. Mitochondrial functions

For mitochondrial isolation, fresh gastrocnemius muscle was rinsed with phosphate-buffered saline and put into ice-cold isolation buffer [0.075 M sucrose, 0.225 M sorbitol, 1 mM EGTA, 0.1% fatty acid (FA)-free BSA and 10 mM Tris–HCl, pH 4]. Tissues were sheared carefully to mince, rinsed to get rid of residual blood and then homogenized in 1 ml isolation buffer per 100 mg tissue. The homogenate was centrifuged at 1000*g* for 5 min at 4°C; the resulting supernatant was decanted and saved. The pellet was washed once with isolation buffer. The supernatant was combined and centrifuged at 9000*g* for 10 min at 4°C. The mitochondrial pellet was washed and centrifuged twice at 15,000*g* for 2 min at 4°C with isolation buffer. Mitochondrial

fractions were used immediately after isolation to determine ATP content with a luciferin-luciferase based bioluminescence assay, using a kit according to the manufacturer's instructions (Beyotime Biotech, Jiangsu, China). Enzyme activities of complex I, II, II+III, IV and CS were measured as described in our previous study [40].

2.7. Quantitative real-time PCR

RNA was extracted from EDL muscle using Trizol (Invitrogen, Singapore) and reverse-transcribed with the ReverTra Ace qPCR RT Kit (FSQ-101; TOYOBO, Osaka, Japan). cDNA was amplified by real-time PCR in a total reaction volume of 20 μl using SYBR Green Real-time PCR Master Mix (QPK-201; TOYOBO, Osaka, Japan). Real-time PCR reactions were cycled in StepOne Real-Time PCR System (Applied Biosystems, California, USA). Primer sets are identified in Table S1. Target gene expression was normalized to endogenous β-actin and expressed as $2^{-\Delta\Delta ct}$ relative to the control group. For mitochondrial DNA (mtDNA) content, total DNA was extracted from skeletal muscle and purified. Approximately 10 ng of DNA was used to quantitative PCR as stated above. β-Actin was used as a nuclear DNA marker. AK140265 were used as mtDNA markers.

2.8. Western blot analysis

The frozen skeletal muscle was pulverized in liquid nitrogen and processed in a 1% Triton X-100 buffer, and homogenates were centrifuged for 10 min at 8000*g* and 4°C. The protein content of the supernatant was quantified using bicinchoninic acid reagents and BSA standards. Proteins were separated by SDS-PAGE and transferred to PVDF membrane and incubated with the primary antibody and horseradish peroxidase-coupled antispecies antibodies. Proteins were visualized by enhanced chemiluminescence and quantified by densitometry. Antibodies recognizing acetylated-p53 and SIRT1 were obtained from Cell Signaling.

2.9. Statistical analysis

Data are reported as the mean±SEM. Comparisons between groups were made using unpaired, two-tailed Student's *t* tests. For time course data, two-way and one-way ANOVA were used where appropriate. A value of $P<.05$ was considered significant.

3. Results

3.1. NAM treatment impairs glucose homeostasis and insulin sensitivity in mice fed regular chow

NAM-treated and control mice were fed regular chow for 8 weeks. Intraperitoneal glucose tolerance tests (IPGTT) were performed to determine the effect of NAM on glucose homeostasis. IPGTT demonstrated that plasma glucose concentrations remained higher in NAM-treated mice than in control mice during 15–90 min after glucose injection, whereas no pronounced difference was observed at the end of the entire experiment (Fig. 1A). The area under the curve for IPGTT was higher for NAM-treated mice than for control mice (Fig. 1C). Insulin tolerance was tested to determine whether this glucose intolerance was associated with systemic insulin resistance. After 8 weeks of NAM treatment, the capacity to reduce blood glucose levels after an injection of insulin was significantly reduced compared with control mice (Fig. 1B and D). NAM also resulted in higher circulating TG and FFA levels compared with control mice (Fig. 1G and H). No changes in fasting blood glucose or insulin level were noted in the NAM-treated mice (Fig. 1E and F). NAM-treated mice did not differ significantly from control mice in body weight, body composition or the weight of fat depots (Fig. 1I–K). Our findings suggest that chronic treatment with NAM results in systemic insulin resistance without obesity in mice fed a regular chow diet.

3.2. NAM treatment leads to increased lipid accumulation and decreased glycogen storage in skeletal muscle

Due to the fact that NAM-induced insulin resistance was not accompanied by obesity, we assessed the lipid content in skeletal muscle and liver. Skeletal muscle TAG levels were higher in NAM-treated mice compared with controls, but this difference was not significant in liver (Fig. 2A). Further, there was a significant decrease in muscular and hepatic glycogen content in NAM-treated mice (Fig. 2B). NAM reduced exogenous FA oxidation and increased TAG esterification in skeletal muscle (Fig. 2C and D). We measured the concentration of the intracellular lipid metabolite DAG in skeletal muscle. We found that NAM led to a significant increase in skeletal muscle DAG content compared with controls (Fig. 2E) but no change in ceramide content (Fig. 2F). We also found that NAM caused a significant muscle loss in EDL (Fig. 2G). The present data demonstrate that NAM alters energy substrate preference in skeletal muscle and reduces the capacity of FA oxidation.

Most metabolic genes implicated in glucose and lipid utilization, including CPT-2, TIGAR, TBC1D1 and PRDM16, were reduced in the NAM-treated mice, while expression of PDK4, LKB1, AMPKα2, Cidea and UCP3, was increased (Fig. 2H–K). Here, we found that NAM increased the transcript levels of Cidea by 9- to 10-fold, PDK4 by 7- to 8-fold and UCP3 by 2-fold in skeletal muscle (Fig. 2H–K), suggesting a series of molecular adaptations to the increase in lipid accumulation. p53-inducible regulator TIGAR inhibits glycolysis and promotes respiration by reducing cellular levels of fructose-2,6-bisphosphat; thus, TIGAR expression improves energy yield from glucose *via* increased respiration [41]. TBC1D1, a member of the TBC1 Rab-GTPase family, regulates both insulin- and contraction-stimulated glucose transport in skeletal muscle [42]. PRDM16 is a transcription regulator leading to the transcription of genes involved in FA oxidation and thermogenesis [43]. mRNA content for CPT-2, TIGAR, TBC1D1 and PRDM16 in skeletal muscle corresponded with the decrease in FA oxidation and glycogen content after NAM treatment (Fig. 2B and C). These results were quite consistent with the skeletal muscle changes in the storage and utilization of triglycerides.

3.3. NAM treatment impairs mitochondrial function and energy production in skeletal muscle

NAM was previously identified as a neuronal nutrient to maintain DNA integrity, phosphatidylserine membrane asymmetry and mitochondrial membrane potential in neurodegenerative disorders [44]. Thus, NAM may be a potential nutrient for mitochondria. To explore the molecular mechanisms by which NAM induces insulin resistance and increases skeletal muscle lipid accumulation, we assayed mitochondrial function in skeletal muscle and the expression of genes implicated in mitochondrial biogenesis and autophagy. Compared to controls, NAM significantly decreased ATP production and mitochondrial complex I, IV activity in skeletal muscle (Fig. 3A and B). Real-time PCR analysis showed that NAM reduced mtDNA content and the expression of genes required for mitochondrial complex IV assembly, such as COX2, SCO1 and SCO2 (Fig. 3C and E). NAM had no significant effects on the transcript levels of genes involved in mitochondrial biogenesis except Tfb2m (Fig. 3D). NAM increased the mRNA expression of NCoR1 and RIP140 (Fig. 3D). These results suggest that chronic NAM treatment impairs mitochondrial function and energy production in skeletal muscle.

Next, we assessed the mRNA levels of autophagy-related genes and the key mitophagy mediators, such as p62, PINK1, PARK2 and NIX [45]. NAM significantly increased the expression of p62, PINK1, PARK2 and NIX (Fig. 3F). In agreement with the changes in mitophagy mediators, NAM significantly increased the expression of FOXO3, Bnip3 and CTSL (Fig. 3G). Also, NAM increased the expression of Beclin-1 and LC-3b, while we found no pronounced changes in ULK1, Atg7, Atg9 and LAMP2 (Fig. 3H). Furthermore, we found that NAM selectively increased the expression of type II myosin, such as IIa, IIb and IIx, and resulted in a nonsignificant reduction in type I myosin (Fig. 3I). As type II myofiber has lower mitochondrial mass than type I myofiber (oxidative muscle fibers), our results are quite consistent with the skeletal muscle data in that NAM impairs mitochondrial function and reduces mtDNA content.

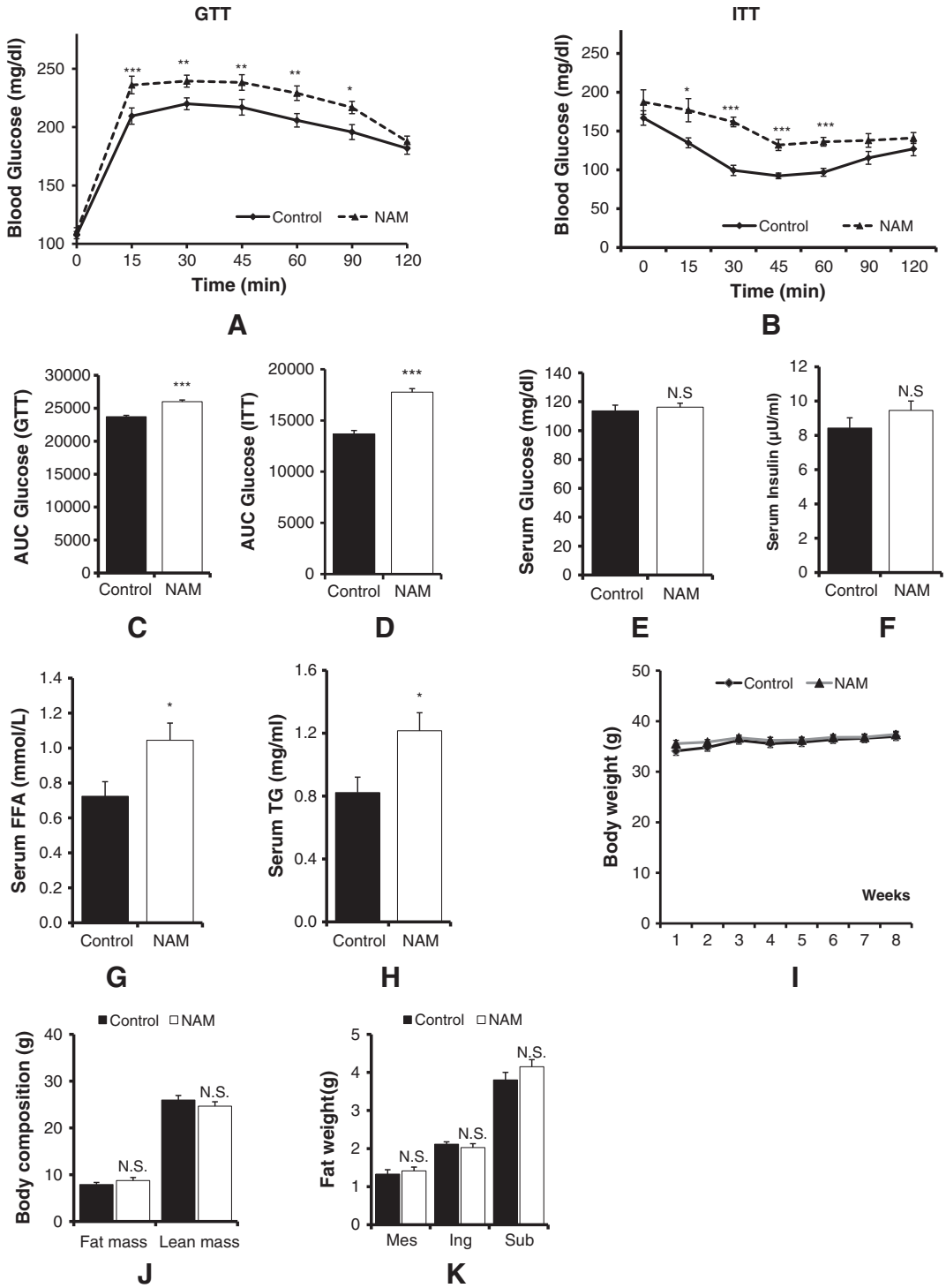


Fig. 1. Chronic NAM treatment induces insulin resistance without obesity in mice. (A and B) Plasma glucose during GTT and ITT (n=8). (C and D) Area under the curves (AUC) for glucose calculated from data in (A) and (B). (E–H) Fasting serum glucose, insulin, FFA and triglyceride (n=8). (I) Body weight of mice fed *ad libitum* treated with NAM for 8 weeks (n=10). (J) Lean and fat body mass in control and NAM mice measured by MRI (n=8). (K) Wet weight of mesenteric (Mes), inguinal (Ing) and subcutaneous (Sub) fat in mice (n=8). *P<.05, **P<.01, ***P<.001 *vs.* Control, N.S: not significant. Mean data are shown with the SE (error bars).

3.4. NAM treatment inhibits SIRT1 expression and its deacetylase activity

NAM inhibits SIRT1 deacetylase activity by binding to a conserved pocket adjacent to NAD+ and blocking NAD+ hydrolysis, rather than by increasing the NAD+ level or [NAD+]/[NADH] ratio. Contrarily, SIRT1 activity in the absence of NAM is enhanced by an increase in the NAD+ level or [NAD+]/[NADH] ratio [46]. Interestingly, 5 mM NAM but not higher concentrations causes SIRT1 activation in fibroblasts, as demonstrated by decreased acetylation level of histone H3 and p53 [5]. Here, we treated rodents with NAM in order to chronically inhibit SIRT1 activity in skeletal muscle. As expected, NAM treatment decreased SIRT1 mRNA and protein expression in skeletal muscle

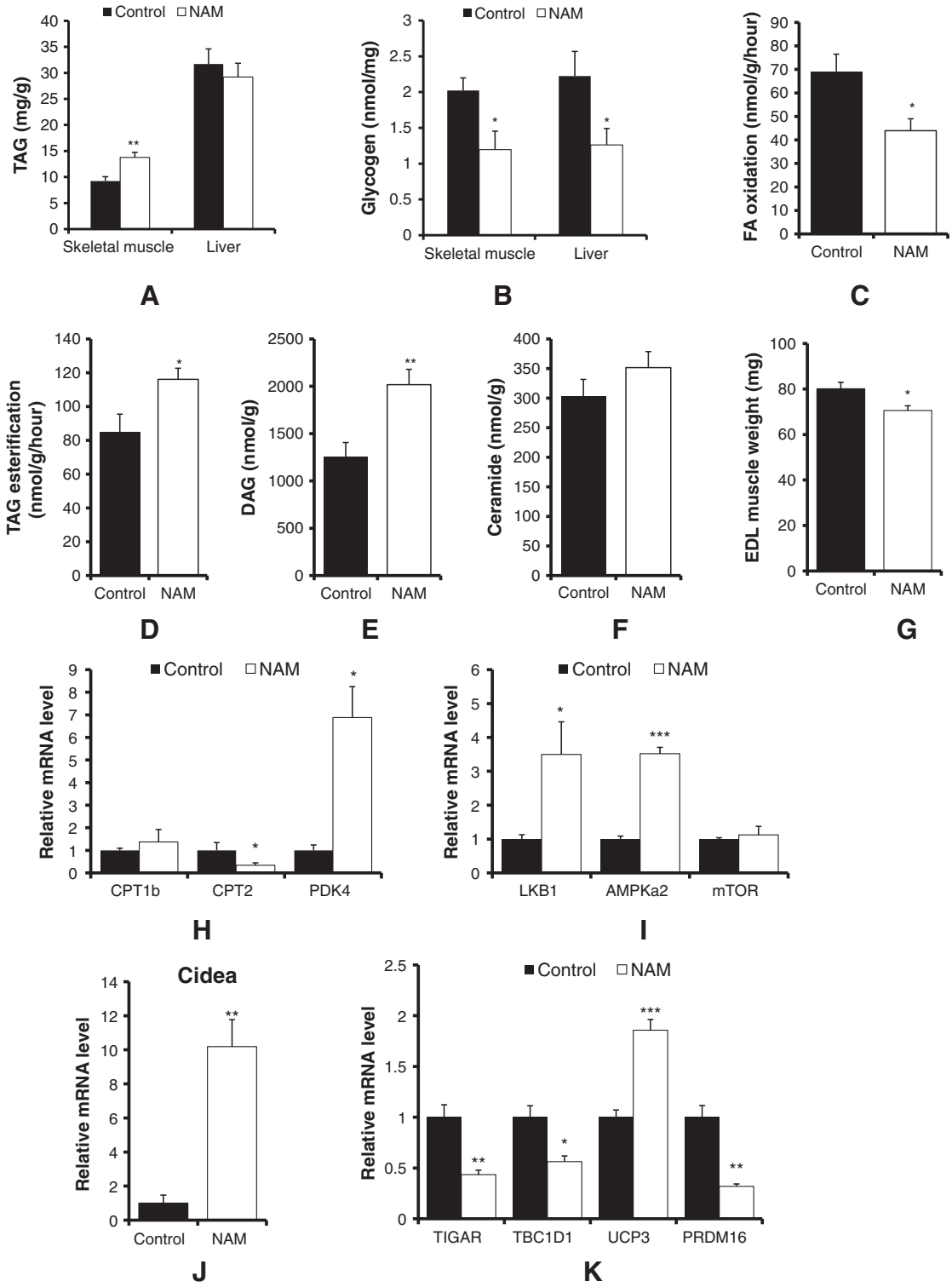


Fig. 2. Chronic NAM treatment increases lipid accumulation in skeletal muscle but not in liver. (A and B) Triacylglycerol (TAG) and glycogen content in skeletal muscle and liver (n=8). (C and D) Exogenous FA oxidation and TAG esterification in skeletal muscle (n=8). (E and F) Total DAG and ceramide in skeletal muscle (n=8). (G) EDL muscle wet weight (n=10). (H–K) Gene expression analysis of glucose and lipid metabolism in skeletal muscle (n=6). Carnitine palmitoyltransterase (CPT-1b and CPT-2), dehydrogenase kinase 4 (PDK4), serine–threonine kinase II (LKB1), AMP-activated protein kinase (AMPKα2), mammalian target of rapamycin (mTOR), cell death-inducing DFFA-like effector A (Cidea), TP53-inducible glycolysis and apoptosis regulator (TIGAR), the Rab-GTPase-activating protein TBC1D1, uncoupling protein 3 (UCP3) and the transcriptional regulator PRDM16. *P<.05, **P<.01, ***P<.001 *vs.* Control. Mean data are shown with the SE (error bars).

(Fig. 4C, E and F). The pronounced increase in acetylation level of p53 was observed in NAM-treated mice (Fig. 4D and F). These results suggest that SIRT1 deacetylase activity, including its expression, was inhibited by NAM in skeletal muscle. Meanwhile, NAM increased [NAD+]/[NADH] ratio (Fig. 4A), whereas the total NAD(H) levels in skeletal muscle were not altered.

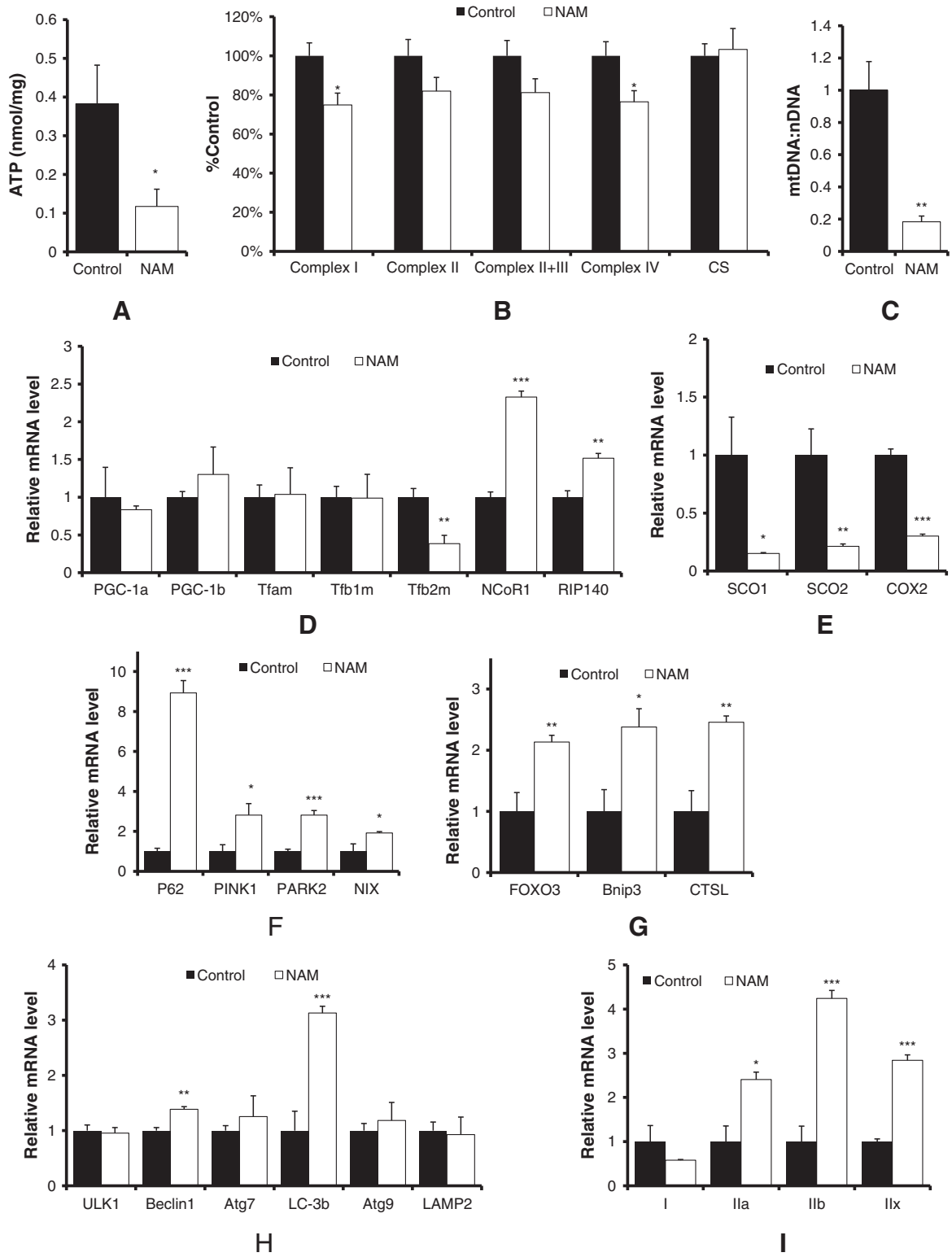


Fig. 3. Chronic NAM treatment impairs ATP production and mitochondrial complex I and IV activities in skeletal muscle. (A) ATP content in the fresh mitochondria isolated from skeletal muscle (n=8). (B) Mitochondrial enzyme activities of complex I, II, II+III, IV and citrate synthase (CS, n=8). (C) mtDNA content relative to nuclear DNA (n=6). (D and E) Gene expression analysis of mitochondrial biogenesis, nuclear receptor corepressors (NCoR1, RIP140) and cytochrome c oxidase assembly (SCO1, SCO2, COX2) in skeletal muscle (n=6). (F–H) Gene expression analysis of mitophagy and autophagy in skeletal muscle (n=6). (I) Gene expression analysis of myosins in skeletal muscle (n=6). *P<.05, **P<.01, ***P<.001 *vs.* Control. Mean data are shown with the SE (error bars).

3.5. RSV treatment protects against diet-induced insulin resistance with obesity

As NAM induced insulin resistance in mice fed regular chow, next we hypothesized that diet-induced insulin resistance may differ from chronic NAM treatment in the regulation of lipid accumulation and that RSV may have beneficial effects on the oxidative substrate selection of skeletal muscle. To test this hypothesis, we treated mice with HFD for 8 weeks and observed that RSV protected against HFD-

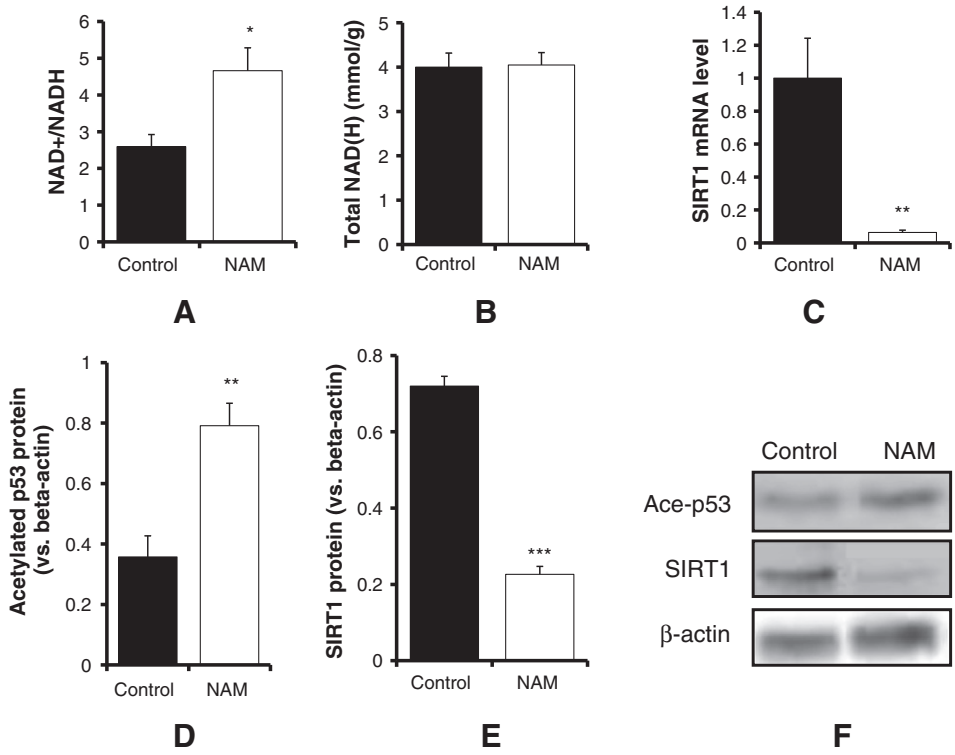


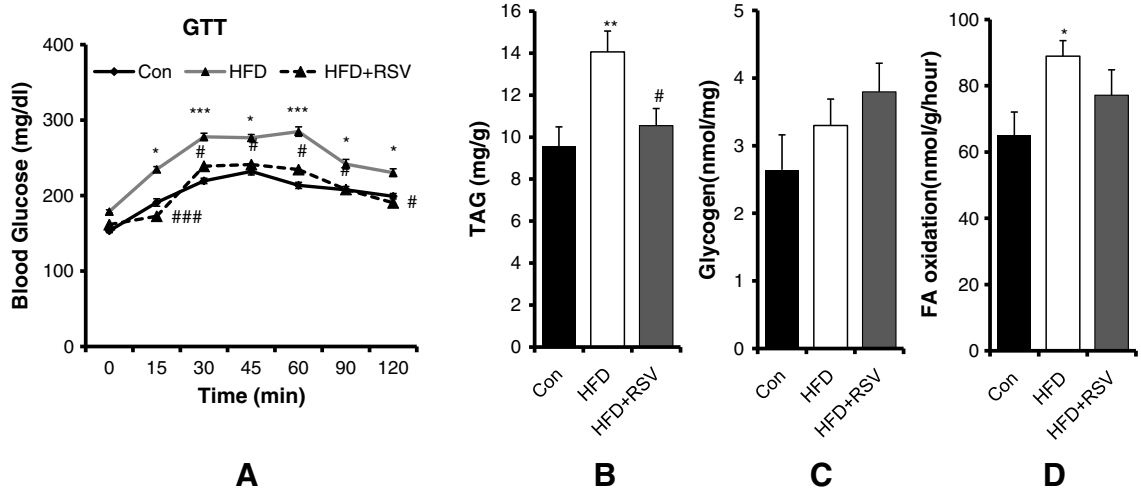
Fig. 4. Chronic NAM treatment inhibits SIRT1 expression and activity. (A) Skeletal muscle NAD+/NADH ratio (n=8). (B) Total NAD(H) concentration in skeletal muscle (n=8). (C) mRNA expression of SIRT1 in skeletal muscle (n=6). (D and E) Intensity of SIRT1 and Ace-p53 was normalized to β-actin (n=4). (F) Representative immunoblot of SIRT1 and acetylated p53 (Ace-p53) in skeletal muscle. *P<.05, **P<.01, ***P<.001 *vs.* Control. Mean data are shown with the SE (error bars).

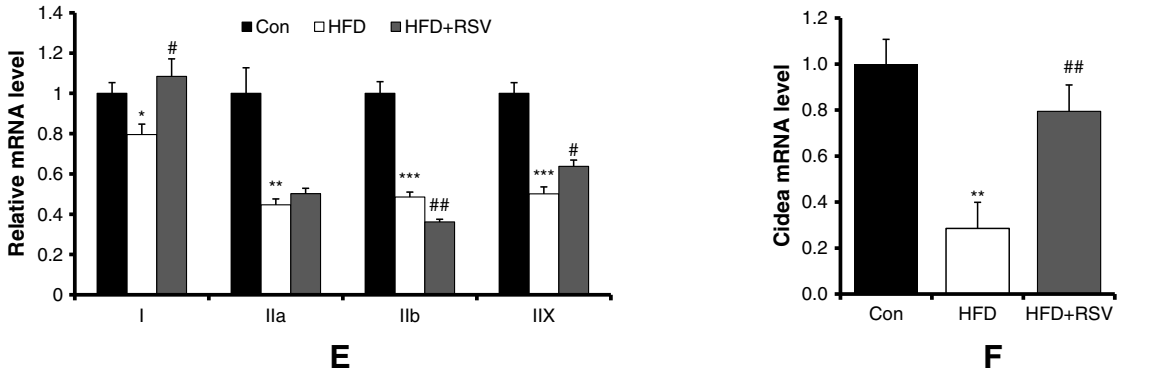
induced insulin resistance and obesity (Fig. 5A, J and K). HFD also increased skeletal muscle TAG content, but this increase was attenuated by RSV (Fig. 5B). Skeletal muscle glycogen content trended to be higher compared with controls, but this difference did not reach statistical significance (Fig. 5C). HFD significantly enhanced FA oxidation (Fig. 5D), suggesting an active response to diets in skeletal muscle. HFD significantly reduced the expression of myosin I, IIa, IIb and IIx, whereas the decrease in myosin I and IIx was rescued by RSV (Fig. 5E). Cidea expression was depressed by HFD, but this reduction was strongly attenuated by RSV (Fig. 5F). In white adipose tissue, Cidea expression correlates positively with lipid deposition and insulin sensitivity [47]. Strikingly, in the current study, skeletal muscle TAG content correlates negatively with mRNA levels of type I myosin and Cidea (Fig. 5H, R=−0.635; Fig. 5I, R=−0.565). Transcript levels of genes involved in mitophagy and autophagy were reduced by HFD, with an 80% reduction in PINK1 and a 40–50% reduction in NIX, FOXO3 and LC-3b (Fig. 5G). These findings suggest that diet-induced insulin resistance is also associated with the increase in skeletal muscle lipid accumulation, whereas chronic NAM treatment differs greatly from HFD in the associated underlying mechanisms.

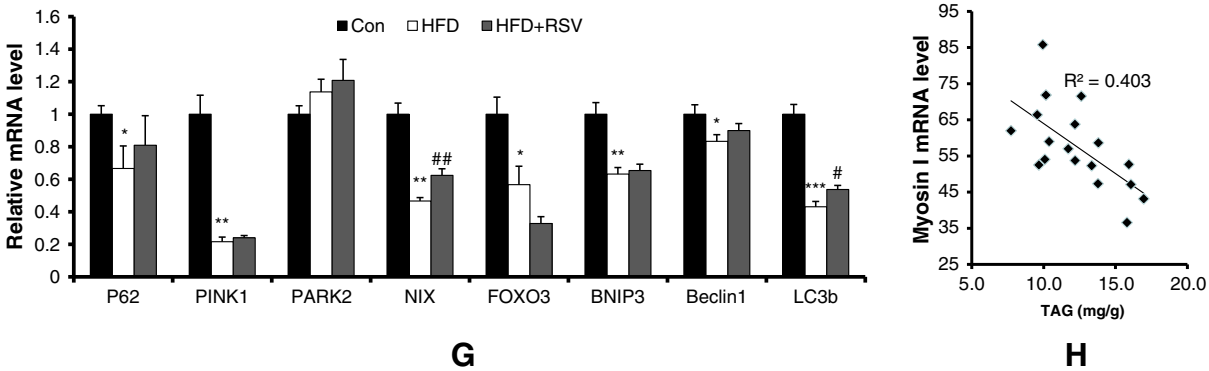
4. Discussion

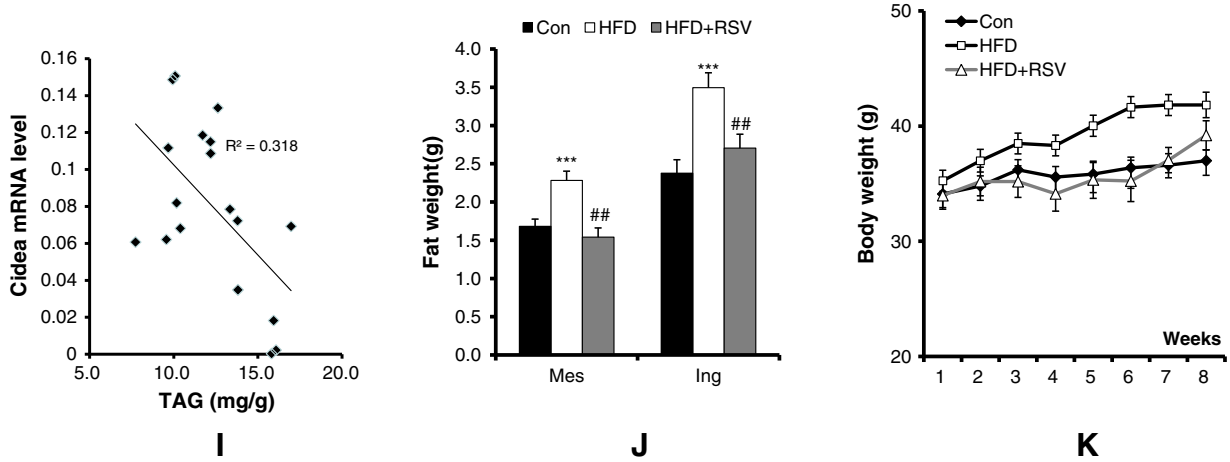
NAM, a form of vitamin B3, is an essential coenzyme in the production of ATP or cellular energy. NAM and NAM derivatives have been proved to preserve neurocognitive function, reduce inflammation and oxidative stress and inhibit cancer's angiogenesis [48–51]. In treatment of obesity and type 2 diabetes, NAM is more effective than nicotinic acid on the regulation of glucose metabolism [10]. However, long-term NAM intake at higher dose induced insulin resistance-related metabolic abnormalities [16]. NR has similar nutritional effects to NAM in ameliorating metabolic disorders. However, NR had a similar toxicity profile to NAM at the highest dose. Target organs of toxicity were liver, kidney, ovaries and testes [52]. Another NAM derivative, isonicotinamide, was found to protect against streptozotocin-induced β-cell damage and diabetes [53]. Administration of both streptozotocin and NAM has been frequently used for inducing type 2 diabetes in rats [54,55]. Therefore, the effects of NAM supplementation on the whole-body metabolism are still problematic. NAM is a precursor not only for NAD+ but also for MNAM. Recent report suggests that increasing NNMT expression or MNAM levels stabilizes SIRT1 protein and results in metabolic benefits [12]. In some other studies, NNMT knockdown in white adipose and liver protects against diet-induced obesity [13]. Plasma MNAM correlates with increased NNMT expression and the degree of insulin resistance [14]. Given these conflicting results regarding NAM supplementation, we sought to investigate whether long-term treatment with NAM at lower dose impairs metabolic homeostasis in mice fed regular chow. Here, we evaluated the role of NAM in the regulation of glucose homeostasis and skeletal muscle lipid metabolism.

Our data showed that NAM treatment (100 mg/kg/day for 8 weeks) induced insulin resistance and skeletal muscle lipid accumulation in mice fed regular chow. This observation clearly demonstrates an adverse effect of NAM intake on insulin sensitivity, conflicting with reported therapeutic effects of NAM in T2DM and obesity [10]. In agreement with our results, there are several studies reporting detrimental metabolic effects of NAM at higher dose in rats and humans, one of which suggests that NAM overload induced an increase in plasma MNAM level, resulting in oxidative stress and insulin resistance [15–17]. It should be noted that our study has examined the effects of NAM only at 100 mg/kg/day. This dosage was much lower than that used for inducing detrimental metabolic effects (4 g/kg/day), whereas the duration was similar to each other [16]. The duration of drug administration in this study was 2-fold longer than


GTT
Con
HFD
HFD+RSV
Blood Glucose (mg/dl)
Time (min)
0 15 30 45 60 90 120
TAG (mg/g)
Glycogen(nmol/mg)
FA oxidation(nmol/g/hour)
Con
HFD
HFD+RSV
A
B
C
D


Con
HFD
HFD+RSV
Relative mRNA level
I
IIa
IIb
IIX
E
Cidea mRNA level
Con
HFD
HFD+RSV
F


Con
HFD
HFD+RSV
Relative mRNA level
P62
PINK1
PARK2
NIX
FOXO3
BNIP3
Beclin1
LC3b
G
Myosin I mRNA level
R² = 0.403
TAG (mg/g)
H


Cidea mRNA level
R² = 0.318
TAG (mg/g)
I
Con
HFD
HFD+RSV
Fat weight(g)
Mes
Ing
J
Body weight (g)
Weeks
K

those in the study reporting therapeutic effects of NAM (4 weeks) [10]. Moreover, we focus on the healthy mice fed regular chow, instead of mice with type 2 diabetes and obesity. We observed that NAM treatment (100 mg/kg/day for 8 weeks) induced glucose intolerance and increased serum lipids. This effect is partially similar to insulin resistance in type 2 diabetes and obesity. However, we found no changes in body weight, fat mass and fasting blood glucose and insulin. Usually, β-cell dysfunction has more contribution to IDDM among nonobese subjects, whereas insulin resistance contributes more to hyperglycemia and type 2 diabetes among obese humans. Actually, insulin resistance was found in nonobese, nondiabetic healthy individuals and nonobese diabetics [56]. The prevalence of insulin resistance in nonobese ethnic group reinforces the importance of maintaining the whole-body energy homeostasis; thus, neither obesity nor excessive lipid storage in adipose tissue is a featured appearance of insulin resistance. Here, we investigated the effects of NAM on lipid content in liver and skeletal muscle. We found no pronounced increase in hepatic lipid storage. Instead, NAM increased lipid content and decreased glycogen content in skeletal muscle. Previous studies demonstrated that nonalcoholic fatty liver disease is tightly associated with insulin resistance in nonobese nondiabetic subjects [57]. Intramuscular triglycerides content was inversely related to insulin sensitivity in nonobese humans [58]. These findings suggest that ectopic lipid deposition in nonadipose tissue protects against obesity rather than insulin resistance. Lipotoxicity represents the elevation of lipids and/or lipid metabolites within blood or tissues with subsequent metabolic disorders and contributes to skeletal muscle insulin resistance [59]. In addition, previous studies reported that insulin resistance decreased activation of glycogen synthase in skeletal muscle in young nonobese patients with type 2 diabetes [60]. However, fast-induced insulin resistance in healthy individuals was associated with glycogen accumulation and increased intramuscular lipid content in skeletal muscle [61], suggesting a great difference from nonobese diabetics in glycogen metabolism. Together, it can be concluded that NAM-induced insulin resistance in nonobese mice was associated with skeletal muscle lipid accumulation. In this work, the possible explanation for skeletal muscle lipid accumulation is that NAM reduced the capacity of exogenous FA oxidation and increased TAG esterification.

Previous studies demonstrated that inhibition of mitochondrial β-oxidation promoted hepatic lipid accumulation and impaired glucose tolerance [62]. Palmitate-induced insulin resistance in muscle was associated with reduced mitochondrial integrity and oxidative capacity. Glucose withdrawal or glycolytic inhibition enhanced insulin sensitivity in muscle cells, suggesting that the dysregulation of mitochondrial substrate availability induced lipid-induced insulin resistance [63]. To gain insight into lipid accumulation and glycogen reduction in skeletal muscle after NAM administration, we explored mitochondrial ATP production, respiratory capacity and the molecular mechanisms involved in mitochondrial biogenesis and autophagy. Here, we show that NAM reduced mitochondrial ATP production and complex I and IV activity, in combination with reduced mtDNA content and transcript levels of genes required for mitochondrial complex IV assembly, such as COX2, SCO1 and SCO2. Further, our results demonstrated that muscle transcript levels of autophagy-related genes (FOXO3, BNIP3, CTSL, BECLIN1 and LC-3b/ATG8), including some specific for mitophagy (P62, PINK1, PARK2 and NIX), were increased after NAM treatment. Recent studies suggested that defective mitophagy led to accumulation of damaged mitochondria, aggravated lipotoxicity and subsequently insulin resistance [64]. Genetic inactivation of signaling adapter p62 led to obesity and insulin resistance, which correlated with reduced energy expenditure and increased adipogenesis [65]. Elevated NIX expression was accompanied by insulin resistance and muscle DAG accumulation in rodents [66]. Enhanced autophagy was frequently reported to reduce lipid accumulation in vascular endothelial cells and hepatocytes [67,68]. These studies proposed that enhanced autophagy reduced lipid storage in tissues, resulting in lower lipotoxicity and improved insulin sensitivity. However, a recent report demonstrated that the levels of autophagy-related genes and proteins in muscle were normal in obesity and type 2 diabetes, suggesting that skeletal muscle autophagy has adapted to hyperglycemia [69]. In another study, intramuscular lipids could contribute to insulin resistance and lead to the induction of autophagy, which was an adaptive response to lipotoxicity [70]. Both of these studies emphasized the adaptability of constitutive autophagy in skeletal muscle *in vivo*. Our findings implied that, although NAM caused mitochondrial dysfunction, the induction of autophagy could be an adaptive response to skeletal muscle lipotoxicity after long-term administration of NAM.

To further understand the differences between NAM-induced and diet-induced insulin resistance, we reproduced diet-induced obesity and insulin resistance and observed the beneficial effects of RSV. Our results indicated that HFD-induced insulin resistance was also related to skeletal muscle lipid accumulation. In contrast to NAM, HFD enhanced the capacity of exogenous FA oxidation, which could be an adaptive response to diet in skeletal muscle. Muscle transcript levels of genes for mitophagy and autophagy were largely repressed by HFD, whereas RSV did not rescue these effects. These findings suggest a different pathway for skeletal muscle lipid accumulation. Previous studies demonstrated that palmitate induced mitophagy deficiency, leading to the accumulation of damaged mitochondria and lipotoxicity [64]. Impaired mitophagy was paralleled by reduced muscle respiratory capacity, lipid accumulation and insulin resistance [71]. Enhanced autophagic flux reduced lipid accumulation in steatotic hepatocytes [67]. It can be concluded that HFD-induced lipid accumulation resulted from the inhibition of autophagy and mitophagy, whereas NAM-induced lipid accumulation is due to impaired FA oxidation and abnormal activation of mitophagy. Anyway, lipid accumulation in skeletal muscle may impair insulin sensitivity, independent of obesity. However, increased muscle lipid storage is often considered not to impair insulin sensitivity but to match fat oxidative capacity optimally when FA availability is elevated by exercise [72]. Previous studies demonstrated that the depletion of Cidea with RNAi markedly elevates lipolysis in human adipocytes, whereas Cidea expression in white adipose tissue increased lipid deposition [47]. Overexpression of Cidea in mouse liver resulted in increased hepatic lipid accumulation and the formation of large lipid droplets [73]. The carboxy-terminal domain of Cidea directs lipid droplet targeting, clustering and TAG accumulation, whereas the amino-terminal domain is required for Cidea-mediated development of enlarged lipid droplets [74]. In this study, HFD-induced lipid accumulation was independent of Cidea expression; instead, Cidea expression was strongly suppressed by HFD. The decrease in Cidea expression may contribute to lipolysis and the adaptive increase in FA oxidation in response to HFD. Compared to

Fig. 5. RSV protects against diet-induced lipid accumulation in skeletal muscle. (A) Plasma glucose during GTT (n=8). (B and C) Triacylglycerol (TAG) and glycogen content in skeletal muscle (n=8). (D) Exogenous FA oxidation in skeletal muscle (n=6). (E–G) Gene expression analysis of myosins, Cidea and the key regulators of mitophagy and autophagy (n=6). (H and I) Negative correlations between skeletal muscle TAG content and the mRNA expression of myosin I and Cidea (n=18). (J) Wet weight of mesenteric (Mes) and inguinal (Ing) fat in mice (n=8). (K) Body weight of mice treated with HFD and RSV for 8 weeks (n=8). $^{*}P$<.05, $^{**}P$<.01, $^{***}P$<.001 *vs.* Control. #P<.05, ##P<.01 *vs.* HFD. Mean data are shown with the SE (error bars).

HFD, NAM strongly increased Cidea mRNA levels, contributing to lipid droplet fusion and the enlargement of lipid droplets [75]. Additionally, we found that NAM selectively increased the mRNA levels of myosin II (IIa, IIb and IIx, fast-twitch fiber) and made a nonsignificant reduction in myosin I (slow-twitch fiber). However, HFD reduced the expression of all of them, suggesting that HFD led to muscle degeneration accompanied by lipid accumulation [76]. A possible explanation for NAM to induce insulin resistance and lipid accumulation could be that muscle fiber-type switching reduced lipid utilization and mitochondrial mass.

Finally, we need point out the potential limitations of the study. Because we observed the nonobese insulin resistance and skeletal muscle lipotoxicity in chow diet-fed mice with NAM administration, we focused on exploring the mechanisms underlying the particular metabolic abnormality. Our data help to clarify whether long-term vitamin B3 supplementation is essential or redundant for general people. Although we reproduced diet-induced insulin resistance as compared to the nonobese insulin resistance and showed the beneficial effects of RSV, the reciprocal actions between NAM, RSV and diet in influencing metabolic balance were not determined. This is a relevant question to the current topic. This study did not explain why lipid accumulation occurs only in skeletal muscle but not in liver. Without comparative study in mice with muscle loss of SIRT1, it is difficult to suggest whether effects of NAM on mitophagy/autophagy are SIRT1 dependent. In addition, NAM is transformed rapidly to NAD+, NADP+ or MNAM *in vivo*. NAD+ and NADP+ are important coenzymes for bioenergetics and MNAM is a novel biomarker for insulin resistance. The plasma levels of NAM and its metabolites, if determined, will make our conclusion more complete. Considering our results conflicted with previous studies reporting the metabolic benefits of NAM, NAM exposure was presumably limited by the comparably low dose and the once-daily administration.

In conclusion, the present results suggest that long-term treatment with NAM, although at lower dose, leads to glucose intolerance and skeletal muscle lipid accumulation in mice fed regular chow. The findings suggest that NAM-mediated metabolic disorders are related to mitochondrial dysfunction and adaptive responses of mitophagy and autophagy. Although HFD has a similar effect to NAM on glucose homeostasis and skeletal muscle lipid metabolism, HFD differs largely from NAM treatment in the effects on mitophagy, autophagy, Cidea and myosin expression. Our study suggests a metabolic risk for healthy individuals who are chronically exposed to vitamin B supplementation. Future studies are needed to determine what dosage for NAM is beneficial to optimally improve or treat specific conditions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2016.07.005.

Disclosure Summary

The authors declare that there is no duality of interest associated with this manuscript.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 31300977), Shanghai Pujiang Program (15PJ032) and the Key Laboratory Construction Project of Adolescent Health Assessment and Exercise Intervention of Ministry of Education, China (No. 40500-541235-14203/004). We thank the students at Prof. Ding's laboratory for the technical support on animal care, biochemical assays, PCR and Western blot. The authors also thank A. Abuliz (Howard Hughes Medical Institute at Yale University School of Medicine) for his very helpful technical guide on GTT, ITT and metabolic analysis.

References

[1] Canto C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, et al. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 2010;11:213–9.
[2] Liang F, Kume S, Koya D. SIRT1 and insulin resistance. Nat Rev Endocrinol 2009;5:367–73.
[3] Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109–22.
[4] Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, North BJ, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 2012;15:675–90.
[5] Jang SY, Kang HT, Hwang ES. Nicotinamide-induced mitophagy: event mediated by high NAD+/NADH ratio and SIRT1 protein activation. J Biol Chem 2012;287:19304–14.
[6] Lee JT, Gu W. SIRT1: regulator of p53 deacetylation. Genes Cancer 2013;4:112–7.
[7] Yamada K, Nonaka K, Hanafusa T, Miyazaki A, Toyoshima H, Tarui S. Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulitis. An observation in nonobese diabetic (NOD) mice. Diabetes 1982;31:749–53.
[8] LeDoux SP, Hall CR, Forbes PM, Patton NJ, Wilson GL. Mechanisms of nicotinamide and thymidine protection from alloxan and streptozocin toxicity. Diabetes 1988;37:1015–9.
[9] Elliott RB, Chase HP. Prevention or delay of type 1 (insulin-dependent) diabetes mellitus in children using nicotinamide. Diabetologia 1991;34:362–5.
[10] Yang SJ, Choi JM, Kim L, Park SE, Rhee EJ, Lee WY, et al. Nicotinamide improves glucose metabolism and affects the hepatic NAD-sirtuin pathway in a rodent model of obesity and type 2 diabetes. J Nutr Biochem 2014;25:66–72.
[11] Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, et al. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 2012;15:838–47.
[12] Hong S, Moreno-Navarrete JM, Wei X, Kikukawa Y, Tzameli I, Prasad D, et al. Nicotinamide *N*-methyltransferase regulates hepatic nutrient metabolism through Sirt1 protein stabilization. Nat Med 2015;21:887–94.
[13] Kraus D, Yang Q, Kong D, Banks AS, Zhang L, Rodgers JT, et al. Nicotinamide *N*-methyltransferase knockdown protects against diet-induced obesity. Nature 2014;508:258–62.
[14] Kannt A, Pfenninger A, Teichert L, Tonjes A, Dietrich A, Schon MR, et al. Association of nicotinamide-*N*-methyltransferase mRNA expression in human adipose tissue and the plasma concentration of its product, 1-methylnicotinamide, with insulin resistance. Diabetologia 2015;58:799–808.
[15] Greenbaum CJ, Kahn SE, Palmer JP. Nicotinamide's effects on glucose metabolism in subjects at risk for IDDM. Diabetes 1996;45:1631–4.
[16] Li D, Tian YJ, Guo J, Sun WP, Lun YZ, Guo M, et al. Nicotinamide supplementation induces detrimental metabolic and epigenetic changes in developing rats. Br J Nutr 2013;110:2156–64.
[17] Zhou SS, Li D, Sun WP, Guo M, Lun YZ, Zhou YM, et al. Nicotinamide overload may play a role in the development of type 2 diabetes. World J Gastroenterol 2009;15:5674–84.
[18] Sun WP, Li D, Lun YZ, Gong XJ, Sun SX, Guo M, et al. Excess nicotinamide inhibits methylation-mediated degradation of catecholamines in normotensives and hypertensives. Hypertens Res 2012;35:180–5.
[19] Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 2006;86:205–43.
[20] Eckardt K, Taube A, Eckel J. Obesity-associated insulin resistance in skeletal muscle: role of lipid accumulation and physical inactivity. Rev Endocr Metab Disord 2011;12:163–72.
[21] Goodpaster BH, Wolf D. Skeletal muscle lipid accumulation in obesity, insulin resistance, and type 2 diabetes. Pediatr Diabetes 2004;5:219–26.
[22] Levin K, Daa SH, Alford FP, Beck-Nielsen H. Morphometric documentation of abnormal intramyocellular fat storage and reduced glycogen in obese patients with type II diabetes. Diabetologia 2001;44:824–33.
[23] Rahimi Y, Camporez JP, Petersen MC, Pesta D, Perry RJ, Jurczak MJ, et al. Genetic activation of pyruvate dehydrogenase alters oxidative substrate selection to induce skeletal muscle insulin resistance; 2014 16508–13.
[24] Divisova J, Kazdova L, Hubova M, Meschisvili E. Relationship between insulin resistance and muscle triglyceride content in nonobese and obese experimental models of insulin resistance syndrome. Ann N Y Acad Sci 2002;967:440–5.
[25] Johannsen DL, Conley KE, Bajpeyi S, Punyanitya M, Gallagher D, Zhang Z, et al. Ectopic lipid accumulation and reduced glucose tolerance in elderly adults are accompanied by altered skeletal muscle mitochondrial activity. J Clin Endocrinol Metab 2012;97:242–50.
[26] Mishra P, Varuzhanyan G, Pham AH, Chan DC. Mitochondrial dynamics is a distinguishing feature of skeletal muscle fiber types and regulates organellar compartmentalization. Cell Metab 2015;22:1033–44.
[27] Stotland A, Gottlieb RA. Mitochondrial quality control: easy come, easy go. Biochim Biophys Acta 2015;1853:2802–11.
[28] Da CS, Parone PA, Lopes VS, Lillo C, McAlonis-Downes M, Lee SK, et al. Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS. Cell Metab 2012;15:778–86.
[29] Hoeks J, Arany Z, Phielix E, Moonen-Kornips E, Hesselink MK, Schrauwen P. Enhanced lipid-but not carbohydrate-supported mitochondrial respiration in

skeletal muscle of PGC-1alpha overexpressing mice. J Cell Physiol 2012;227:1026–33.
[30] Qi Z, Ding S. Transcriptional regulation by nuclear corepressors and PGC-1alpha: implications for mitochondrial quality control and insulin sensitivity. PPAR Res 2012;2012:348245.
[31] Yamamoto H, Williams EG, Mouchiroud L, Canto C, Fan W, Downes M, et al. NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. Cell 2011;147:827–39.
[32] Masiero E, Agatea L, Mammucari C, Blaauw B, Loro E, Komatsu M, et al. Autophagy is required to maintain muscle mass. Cell Metab 2009;10:507–15.
[33] Farah BL, Landau DJ, Sinha RA, Brooks ED, Wu Y, Fung SY, et al. Induction of autophagy improves hepatic lipid metabolism in glucose-6-phosphatase deficiency. J Hepatol 2016;64:370–9.
[34] Torisu K, Singh KK, Torisu T, Lovren F, Liu J, Pan Y, et al. Intact endothelial autophagy is required to maintain vascular lipid homeostasis. Aging Cell 2016;15:187–91.
[35] Zhou SS, Zhou Y. Excess vitamin intake: an unrecognized risk factor for obesity. World J Diabetes 2014;5:1–13.
[36] Jurczak MJ, Lee HY, Birkenfeld AL, Jornayvaz FR, Frederick DW, Pongratz RL, et al. SGLT2 deletion improves glucose homeostasis and preserves pancreatic beta-cell function. Diabetes 2011;60:890–8.
[37] Wendel AA, Li LO, Li Y, Cline GW, Shulman GI, Coleman RA. Glycerol-3-phosphate acyltransferase 1 deficiency in Ob/Ob mice diminishes hepatic steatosis but does not protect against insulin resistance or obesity. Diabetes 2010;59:1321–9.
[38] Greenberg CC, Meredith KN, Yan L, Brady MJ. Protein targeting to glycogen overexpression results in the specific enhancement of glycogen storage in 3T3-L1 adipocytes. J Biol Chem 2003;278:30835–42.
[39] Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. J Physiol 2005;565:537–46.
[40] Sun L, Shen W, Liu Z, Guan S, Liu J, Ding S. Endurance exercise causes mitochondrial and oxidative stress in rat liver: effects of a combination of mitochondrial targeting nutrients. Life Sci 2010;86:39–44.
[41] Li H, Jogl G. Structural and biochemical studies of TIGAR (TP53-induced glycolysis and apoptosis regulator). J Biol Chem 2009;284:1748–54.
[42] An D, Toyoda T, Taylor EB, Yu H, Fujii N, Hirshman MF, et al. TBC1D1 regulates insulin- and contraction-induced glucose transport in mouse skeletal muscle. Diabetes 2010;59:1358–65.
[43] Villanueva CJ, Vergnes L, Wang J, Drew BG, Hong C, Tu Y, et al. Adipose subtype-selective recruitment of TLE3 or Prdm16 by PPARgamma specifies lipid storage versus thermogenic gene programs. Cell Metab 2013;17:423–35.
[44] Chong ZZ, Lin SH, Maiese K. The NAD+ precursor nicotinamide governs neuronal survival during oxidative stress through protein kinase B coupled to FOXO3a and mitochondrial membrane potential. J Cereb Blood Flow Metab 2004;24:728–43.
[45] Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 2010;12:119–31.
[46] Milner J. Cellular regulation of SIRT1. Curr Pharm Des 2009;15:39–44.
[47] Puri V, Ranjit S, Konda S, Nicoloro SM, Straubhaar J, Chawla A, et al. Cidea is associated with lipid droplets and insulin sensitivity in humans. Proc Natl Acad Sci U S A 2008;105:7833–8.
[48] Rennie G, Chen AC, Dhillon H, Vardy J, Damian DL. Nicotinamide and neurocognitive function. Nutr Neurosci 2015;18:193–200.
[49] Lappas M, Permezel M. The anti-inflammatory and antioxidative effects of nicotinamide, a vitamin B(3) derivative, are elicited by FoxO3 in human gestational tissues: implications for preterm birth. J Nutr Biochem 2011;22:1195–201.
[50] Choi HE, Choi JH, Lee JY, Kim JH, Kim JH, Lee JK, et al. Synthesis and evaluation of nicotinamide derivative as anti-angiogenic agents. Bioorg Med Chem Lett 2013;23:2083–8.
[51] Weiss R, Schilling E, Grahnert A, Kolling V, Dorow J, Ceglarek U, et al. Nicotinamide: a vitamin able to shift macrophage differentiation toward macrophages with restricted inflammatory features. Innate Immun 2015;21:813–26.
[52] Conze DB, Crespo-Barreto J, Kruger CL. Safety assessment of nicotinamide riboside, a form of vitamin B3. Hum Exp Toxicol 2016 [Epub ahead of print].
[53] Fukaya M, Tamura Y, Chiba Y, Tanioka T, Mao J, Inoue Y, et al. Protective effects of a nicotinamide derivative, isonicotinamide, against streptozotocin-induced beta-cell damage and diabetes in mice. Biochem Biophys Res Commun 2013;442:92–8.
[54] Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med (Maywood) 2012;237:481–90.
[55] Wu MS, Liang JT, Lin YD, Wu ET, Tseng YZ, Chang KC. Aminoguanidine prevents the impairment of cardiac pumping mechanics in rats with streptozotocin and nicotinamide-induced type 2 diabetes. Br J Pharmacol 2008;154:758–64.
[56] Araneta MR, Wingard DL, Barrett-Connor E. Type 2 diabetes and metabolic syndrome in Filipina-American women : a high-risk nonobese population. Diabetes Care 2002;25:494–9.
[57] Musso G, Gambino R, Bo S, Uberti B, Biroli G, Pagano G, et al. Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome? A cross-sectional comparison with adult treatment panel III criteria in nonobese nondiabetic subjects. Diabetes Care 2008;31:562–8.
[58] Perseghin G, Scifo P, Pagliato E, Battezzati A, Benedini S, Soldini L, et al. Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. J Clin Endocrinol Metab 2001;86:3188–96.
[59] Chow L, From A, Seaquist E. Skeletal muscle insulin resistance: the interplay of local lipid excess and mitochondrial dysfunction. Metabolism 2010;59:70–85.
[60] Vaag A, Henriksen JE, Beck-Nielsen H. Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. J Clin Invest 1992;89:782–8.
[61] Vendelbo MH, Clasen BF, Treebak JT, Moller L, Krusenstjerna-Hafstrom T, Madsen M, et al. Insulin resistance after a 72-h fast is associated with impaired AS160 phosphorylation and accumulation of lipid and glycogen in human skeletal muscle. Am J Physiol Endocrinol Metab 2012;302:E190–200.
[62] Bhatia H, Pattnaik BR, Datta M. Inhibition of mitochondrial beta-oxidation by miR-107 promotes hepatic lipid accumulation and impairs glucose tolerance *in vivo*. Int J Obes (Lond) 2016;40:861–9.
[63] Lipina C, Macrae K, Suhm T, Weigert C, Blachnio-Zabielska A, Baranowski M, et al. Mitochondrial substrate availability and its role in lipid-induced insulin resistance and proinflammatory signaling in skeletal muscle. Diabetes 2013;62:3426–36.
[64] Yang S, Xia C, Li S, Du L, Zhang L, Zhou R. Defective mitophagy driven by dysregulation of rheb and KIF5B contributes to mitochondrial reactive oxygen species (ROS)-induced nod-like receptor 3 (NLRP3) dependent proinflammatory response and aggravates lipotoxicity. Redox Biol 2014;3:63–71.
[65] Lee SJ, Pfluger PT, Kim JY, Nogueiras R, Duran A, Pages G, et al. A functional role for the p62-ERK1 axis in the control of energy homeostasis and adipogenesis. EMBO Rep 2010;11:226–32.
[66] Mughal W, Nguyen L, Pustylnik S, Da SRS, Piotrowski S, Chapman D, et al. A conserved MADS-box phosphorylation motif regulates differentiation and mitochondrial function in skeletal, cardiac, and smooth muscle cells. Cell Death Dis 2015;6:e1944.
[67] Zhou SW, Zhang M, Zhu M. Liraglutide reduces lipid accumulation in steatotic L02 cells by enhancing autophagy. Mol Med Rep 2014;10:2351–7.
[68] Kim HS, Montana V, Jang HJ, Parpura V, Kim JA. Epigallocatechin gallate (EGCG) stimulates autophagy in vascular endothelial cells: a potential role for reducing lipid accumulation. J Biol Chem 2013;288:22693–705.
[69] Kruse R, Vind BF, Petersson SJ, Kristensen JM, Hojlund K. Markers of autophagy are adapted to hyperglycaemia in skeletal muscle in type 2 diabetes. Diabetologia 2015;58:2087–95.
[70] De Stefanis D, Mastrocola R, Nigro D, Costelli P, Aragno M. Effects of chronic sugar consumption on lipid accumulation and autophagy in the skeletal muscle. Eur J Nutr 2015 [Epub ahead of print].
[71] Drew BG, Ribas V, Le JA, Henstridge DC, Phun J, Zhou Z, et al. HSP72 is a mitochondrial stress sensor critical for Parkin action, oxidative metabolism, and insulin sensitivity in skeletal muscle. Diabetes 2014;63:1488–505.
[72] Newsom SA, Schenk S, Li M, Everett AC, Horowitz JF. High fatty acid availability after exercise alters the regulation of muscle lipid metabolism. Metabolism 2011;60:852–9.
[73] Zhou L, Xu L, Ye J, Li D, Wang W, Li X, et al. Cidea promotes hepatic steatosis by sensing dietary fatty acids. Hepatology 2012;56:95–107.
[74] Christianson JL, Boutet E, Puri V, Chawla A, Czech MP. Identification of the lipid droplet targeting domain of the Cidea protein. J Lipid Res 2010;51:3455–62.
[75] Barneda D, Planas-Iglesias J, Gaspar ML, Mohammadyani D, Prasannan S, Dormann D, et al. The brown adipocyte protein CIDEA promotes lipid droplet fusion *via* a phosphatidic acid-binding amphipathic helix. Elife 2015;4.
[76] Ickin GM, Guven BA, Yavuz O, Hismiogullari AA. Histopathological changes in rat pancreas and skeletal muscle associated with high fat diet induced insulin resistance. Biotech Histochem 2015;90:495–505.