High fat diet induced disturbances of energy metabolism

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Chapter 5: The iminosugar AMP-DNM reduces body weight by decreasing food intake and increasing fat oxidation in ob/ob mice

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Abstract

Obesity and its associated conditions such as type 2 diabetes are a major cause of morbidity and mortality. The iminosugar AMP-DNM improves insulin sensitivity in rodent models of insulin resistance and type 2 diabetes. In the current study we characterized the impact of AMP-DNM on substrate oxidation patterns, food intake and body weight gain in obese mice. Eight ob/ob mice treated with 100 mg/kg/day AMP-DNM mixed in the food and eight control ob/ob mice were placed in metabolic cages during the first, third and fifth week of the experiment for measurement of substrate oxidation rates, energy expenditure, activity and food intake. Mice were sacrificed after six weeks of treatment. Initiation of treatment with AMP-DNM resulted in a rapid increase in fat oxidation by 129 % ($p=0.05$), a decrease in carbohydrate oxidation by 35 % (p 0.01) and a reduction in food intake by ~26 % ($p<0.01$) as well as energy expenditure by 12 % ($p<$ 0.03) compared to the control mice. Treatment with AMP-DNM decreased hepatic triglyceride content by 66 % (p <0.01) and increased hepatic CPT-1a expression, which was in line with the elevated whole body fat oxidation rate. AMP-DNM treatment increased the plasma levels of the appetite regulating peptide YY (PYY) compared to control mice, which is in accordance with the lower food intake. Treatment with AMP-DNM rapidly reduces food intake and increases fat oxidation, associated with an increase in PYY levels, resulting in improvement of the obese phenotype. These features of AMP-DNM, together with its insulin-sensitizing capacity, make it an attractive candidate drug for the treatment of obesity and its associated metabolic derangements.

Introduction

As the incidence of obesity and its associated morbidities such as type 2 diabetes increases, the search for new treatment modalities to combat these conditions continues. Decreasing caloric intake and increasing physical activity are effective in improving obesity, hypertension, insulin resistance and dyslipidemia (55;139;149;266). However, the positive effect of these dietary and life style interventions is often short lasted, because permanent changes in life style and diet are hard to achieve (37;60). Moreover, weight reduction obtained by low caloric diets induces adaptations in energy metabolism, resulting in a lower resting energy expenditure (80;260). If caloric intake is subsequently increased again, fat mass may rapidly increase (230;248). The emphasis in the treatment of those patients that fail to acquire or maintain weight loss should therefore be on pharmacological interventions, aimed at persistent weight loss and the reduction of obesity-associated pathologies.

Obesity and type 2 diabetes mellitus are associated with diminished responsiveness to insulin. Insulin resistance leads to impaired glucose and fat homeostasis and results in damage of organs such as the liver and pancreas due to exposure to inappropriately high insulin, glucose and fatty acid concentrations (13;173). Moreover, obesity may have an effect on substrate oxidation patterns. In lean individuals, glucose is preferentially oxidized in the fed state and fat in the fasted state. This ability to switch between carbohydrate and fat oxidation, in relation to nutritional status is referred to as metabolic flexibility and this flexibility is impaired in the obese, insulin resistant state (47). Reduced fat oxidation contributes to ectopic fat accumulation for instance in liver and muscle, further reducing insulin sensitivity (267). Improvement of insulin sensitivity is therefore essential for reduction of the negative consequences of obesity.

Ectopic lipid accumulation results in reduced responsiveness to insulin by the formation of specific lipid metabolites that interfere with insulin signaling (233). Sphingolipids are one class of lipids involved in the induction of insulin resistance. The simplest sphingolipid, ceramide, is formed from palmitoyl-CoA and serine by the enzyme serine-palmitoyltransferase and the availability of palmitoyl-CoA is rate limiting for ceramide synthesis (162). From ceramide, glycosphingolipids and subsequently the more complex gangliosides can be formed. Increased availability of the free fatty acid palmitate and low grade inflammation in obesity enhance the synthesis of sphingolipids and gangliosides (105;142). Ceramide interferes with insulin signaling at the level of PKB-Akt, reducing the metabolic actions of insulin (105;142). The ganglioside GM3, when present in abundance, is thought to hamper phosphorylation of the insulin receptor, resulting in reduced responsiveness to insulin (124;234).

Reduction of sphingolipid synthesis restores insulin sensitivity in mouse models for insulin resistance and type 2 diabetes (1;281). The iminosugar N-(5-

adamantane-1-yl-methoxy-pentyl)-deoxynojirimycin (AMP-DNM) reduces glucosylceramide and subsequent ganglioside synthesis and improves both peripheral and hepatic insulin sensitivity (1). Subsequent studies showed that this drug also improves adipocyte function, reduces inflammation and ameliorates hepatic steatosis in insulin resistant genetically obese ob/ob mice (17). In these studies, a reduction in body weight gain upon treatment with a relatively high dose of 100 mg/kg/day AMP-DNM was noted (17;253). The cause of this reduced body weight gain and the course of the metabolic effects of AMP-DNM administration in time have not been reported so far. To monitor the kinetics of the effect of AMP-DNM on metabolism in ob/ob mice we have now performed an investigation using a setup of computerized metabolic cages, which allows in depth analysis of the effects of the drug on substrate oxidation, food intake, energy homeostasis and activity. In this study we show that treatment with AMP-DNM rapidly increases fat oxidation, reduces carbohydrate oxidation and decreases food intake. These changes result in a sustained lower body weight in the AMP-DNM treated animals.

Methods

Animals, diets and indirect calorimetry

Experiments were all approved by the ethics committee for animal experiments of the Academic Medical Center or Leiden University Medical Center. Leptin deficient ob/ob mice (C57Bl/6J background), 6 weeks old, were purchased from Charles River Laboratories (Maastricht, the Netherlands). Before start of the experiments, the animals were housed in a temperature controlled room on a 12:12-h light-dark cycle for two weeks. They were fed ad libitum with rodent AM-II chow (Arie Blok Diervoeders, Woerden, the Netherlands), containing 24.8% crude protein, 6.6% crude fat (0.018% wt/wt cholesterol), 3.6% crude fiber and 4.5% minerals.

In the first experiment 16 mice were subjected to individual indirect calorimetry measurements (Comprehensive Laboratory Animal Monitoring System, Columbus Instruments, Columbus Ohio, US) for 4 consecutive days during week 1, 3 and 5 of the experiment. A period of 24 hours was included at the start of the experiment to allow acclimatization of the animals to the cages and the single housing. In the first week, after 38 hours of basal measurements, 8 mice were switched to food containing AMP-DNM at a dose of approximately 100 mg/kg body weight/day. Analyzed parameters included real time food intake and activity. Oxygen consumption (VO2) and carbon dioxide production rates (VCO2) were recorded at 7 minute intervals. Respiratory exchange ratio (RER) as a measure for metabolic substrate choice was calculated using the following formula as the ratio between VCO2 and VO2. Carbohydrate and fat oxidation rates were calculated according to Peronnet and Massicotte (190). Total energy expenditure was calculated from the sum of carbohydrate and fat oxidation. Activity was monitored as infrared beam breaks in both X and Y axis.

Body weight was measured at the beginning of the first, third and fifth week and at the end of the experiment. Temperature was measured rectally and blood glucose concentration was determined with a hand held meter (Accu-Check, Roche Diagnostics, Mannheim, Germany) at the end of the first, third and fifth week upon exiting the metabolic cages. On day 37 (week 6), mice were weighed and blood glucose and HbA1C concentrations (A1Cnow, Metrika Inc, Sunnyvale, CA) were determined. Subsequently mice were fasted for four hours and anesthetized by ip. injection with a combination of 6.25 mg/kg acetylpromazine (Sanofi Santé Nutrition Animale, Libourne Cedex, France) 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands). Tissues were quickly removed and immediately placed in liquid N2 and stored at -80°C until further analysis. EDTA plasma was stored at -20ºC.

In the second experiment 12 ob/ob mice were fasted for 4 hours. Subsequently, a single dose of 100 mg/kg body weight AMP-DNM was administered to 6 mice by gavage. Six control mice received vehicle (water) by gavage. Four hours later, mice were anesthetized and blood and tissues were handled as in the first experiment.

Plasma and tissue biochemical analysis

Plasma insulin concentrations were determined by ELISA (Crystal Chem Inc, USA). Colorimetric enzymatic kits were used for the measurements of plasma and liver triglyceride concentrations (Human, Wiesbaden, Germany) and plasma free fatty acid (FFA) concentrations (Wako Chemicals GmbH, Neuss, Germany). Total plasma PYY concentrations (PYY 1-36 and PYY 3-36) were measured using a commercially available kit (Phenix pharmaceuticles, Burlingame, CA, USA). To correlate liver lipid values, the protein content of the liver was measured using the BCA method (Pierce, Perbio Science, Etten-Leur, the Netherlands).

For the analysis of ceramide and glucosylceramide, lipids from 50 µl of 4 times diluted liver homogenate were extracted according to Folch, followed by deacylation in 500 µl 0.1 mol/L NaOH in methanol using a microwave oven (CEM microwave Solids/Moisture System SAM-155). The deacylated lipids were derivatised with O-phtaldehyde reagent and separated by high performance liquid chromatography (HPLC).

Part of the EDTA blood from the second experiment was directly mixed with 1M perchloric acid and stored on ice for at least 10 minutes. After centrifugation the supernatant was neutralized using 2M KOH, 0.5M 2-(N-morpholino)ethanesulfonic acid. Metabolite concentrations were measured in the neutralized supernatant after removal of $KClO_4$. Blood glucose was measured using hexokinase and glucose-6-phosphate dehydrogenase. Pyruvate was

measured using lactate dehydrogenase. For lactate, we used lactate dehydrogenase and glutamate pyruvate transaminase. Beta-hydroxybutyrate was measured using beta-hydroxybutyrate dehydrogenase.

Gene expression in liver and muscle

Total RNA was extracted from approximately 50 mg frozen tissues using Trizol reagent (Invitrogen, Breda, the Netherlands). For cDNA synthesis RNA was treated with RQ1 RNase-free DNase (Promega, Leiden, The Netherlands) and reverse transcribed with SuperScript II Reverse Transcriptase and random hexamers (Invitrogen, Breda, The Netherlands). The real time PCR measurement of individual cDNAs was performed on a Bio-Rad MyiQ Single-Color Real-Time PCR Detection System using the Bio-Rad iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). PCR primers were designed on the basis of Primer Express 1.7 software (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). The expression data were normalized by calculating the ratio with cyclophilin B (Ppib), a housekeeping gene.

Statistical analysis

Data are represented as mean±sd. Statistical analysis for the metabolic cage data were performed on 12 hour averages per parameter, based on the light-dark cycle, apart from total accumulated food intake. Data were generated for the light period between 07:00 and 19:00 and for the dark period between 19:00 and 07:00.

Normality checks were performed and in case of normal distribution of the data, comparisons were made using unpaired t-tests. In all other cases Mann-Whitney tests were used. Significance was set at $p<0.05$.

Results

Exposure to AMP-DNM rapidly increases fat oxidation and decreases carbohydrate oxidation

Leptin-deficient ob/ob mice (n=8) were exposed to 100 mg AMP-DNM/kg bodyweight/day mixed in food for 5 weeks. In parallel, control ob/ob mice (n=8) received identical food without AMP-DNM. In accordance with earlier studies (1;103;281), AMP-DNM was well tolerated and caused no overt side effects. Prior to treatment initiation, respiratory exchange ratio (RER), carbohydrate and fatty acid oxidation rates, energy expenditure and food intake were comparable in both randomized groups.

Exposure to AMP-DNM rapidly decreased the RER (dark period: AMP-DNM 0.88±0.07 vs control 0.95±0.05, p=0.03; light period: AMP-DNM 0.84±0.08 vs control 0.92±0.06, p=0.04), indicating an increase in fat to carbohydrate oxidation ratio (Table 1). This was reflected in a significantly higher absolute fat oxidation rate in AMP-DNM treated animals, both during the dark period (AMP-

DNM 0.16±0.09 vs control 0.07±0.06 kcal/hr, p=0.05) and the light period (AMP-DNM 0.19±0.10 vs control 0.10±0.08 kcal/hr, p=0.08) (Fig. 1D, Table 1). The high fat oxidation rate in the treated animals was maintained during the third (dark period: AMP-DNM 0.18±0.0 kcal/hr; light period: AMP-DNM 0.19±0.06) and fifth week (dark period: AMP-DNM 0.15±0.06 kcal/hr; light period: AMP-DNM 0.16±0.05) of treatment (Table 1).

Table 1: Substrate oxidation rates, energy expenditure and food intake during 5 weeks of AMP-DNM treatment: Values are presented as mean ± SD for eight mice in each group. RER=Respiratory Exchange Ratio, FA = fat oxidation, CO = carbohydrate oxidation, EE = total energy expenditure. Values for food intake are the total grams of food consumed during the final 48 hours of each period in the metabolic cages. RER, glucose and fat oxidation rates and energy expenditure are the mean values of all measurements during either the dark (NRER, NFA, NCO, NEE) or light period (DRER, DFA, DCO, DEE) in the metabolic cages.

	Week 1		Week 3		Week 5	
	AMP-DNM	control	AMP-DNM	control	AMP-DNM	control
NRER	0.88 ± 0.07	0.95 ± 0.05*	0.84 ± 0.05	0.93 ± 0.05*	0.86 ± 0.05	0.87 ± 0.04
DRER	0.84 ± 0.08	0.92 ± 0.06*	0.86 ± 0.04	0.93 ± 0.05*	0.90 ± 0.04	0.90 ± 0.04
NFA (kcal/h)	0.16 ± 0.09	0.07 ± 0.06*	0.18 ± 0.0	0.10 ± 0.07*	0.15 ± 0.06	0.17 ± 0.07
DFA (kcal/h)	0.19 ± 0.10	0.10 ± 0.08	0.19 ± 0.06	0.10 ± 0.07*	0.16 ± 0.05	0.20 ± 0.07
NCO (kcal/h)	0.28 ± 0.12	0.43 ± 0.08*	0.26 ± 0.07	0.42 ± 0.08*	0.37 ± 0.09	0.43 ± 0.10
DCO (kcal/h)	0.20 ± 0.10	0.34 ± 0.09*	0.20 ± 0.08	0.39 ± 0.08*	0.25 ± 0.08	0.34 ± 0.09
NEE (kcal/h)	0.44 ± 0.06	0.50 ± 0.05*	0.43 ± 0.06	0.52 ± 0.04*	0.52 ± 0.08	0.60 ± 0.05*
DEE (kcal/h)	0.39 ± 0.05	0.45 ± 0.04*	0.40 ± 0.07	0.45 ± 0.03*	0.41 ± 0.08	0.54 ± 0.05*
EI (g/48h)	8.02 ± 0.61	10.9 ± 1.47*	8.07 ± 1.18	11.83 ± 1.08*	8.39 ± 1.13	9.88 ± 1.72*

In agreement with the lower RER, carbohydrate oxidation rate was markedly lower in AMP-DNM treated animals during the first week (dark period: AMP-DNM 0.28 ± 0.12 vs control 0.43 ± 0.08 kcal/hr, p=0.01; light period: AMP-DNM 0.20 ± 0.34 vs control 0.34 ± 0.09 kcal/hr, p=0.01) and third week (dark period: AMP-DNM 0.26 ± 0.07 vs control 0.42 ± 0.08 kcal/hr, p=0.01; light period: AMP-DNM 0.20 ± 0.08 vs control 0.39 ± 0.08 kcal/hr, p<0.01) of the experiment. During the fifth week of the experiment, relative substrate oxidation rates were no longer different between the treated and control animals. Dark period and light period RER did not differ significantly between treated and control animals (dark period: AMP-DNM 0.86 ± 0.05 vs control 0.87 ± 0.04, p=1.00; light period:

AMP-DNM 0.90 ± 0.04 vs control 0.90 ± 0.04, p=0.73) and absolute fat oxidation and glucose oxidation rates were similar in both groups (Table 1). Probably, this was due to an aging and obesity related increase in the fat oxidation rate in untreated animals and an increase in glucose oxidation rate in the AMP-DNM treated animals between week 3 and 5 of the experiment. From two weeks of treatment onwards, non-fasted plasma glucose levels were significantly lower in the AMP-DNM treated animals. The improved glucose homeostasis was reflected in lower glycated haemoglobin (HbA1C) concentrations in the treated animals (Table 3), confirming previous data (1;17;253).

Exposure to AMP-DNM decreases food intake and total energy expenditure

Concomitantly with the changes in substrate oxidation patterns, food intake decreased after initiation of treatment (Table 1). Total caloric intake was lower in treated compared to control animals in the first (calories consumed during 48 hrs: AMP-DNM 8.0 ± 0.6 vs control 10.9 ± 1.5 kcal/hr, p<0.01), third (calories consumed during 48 hrs: AMP-DNM 8.1 ± 1.2 vs control 11.8 ± 1.1 kcal/hr, p<0.01) and the fifth week of treatment (calories consumed during 48 hrs: AMP-DNM 8.4 ± 1.1 vs control 9.9 ± 1.7 kcal/hr, p0.04, Table 1).

Total energy expenditure was lower in treated animals during the first (dark period: AMP-DNM 0.44 ± 0.06 vs control 0.50 ± 0.05 kcal/hr, p=0.03; light period: AMP-DNM 0.39 ± 0.05 vs control 0.45 ± 0.04 kcal/hr, p=0.02)(Fig. 1B), third (dark period: AMP-DNM 0.43 ± 0.06 vs control 0.52 ± 0.05 kcal/hr, p<0.01; light period AMP-DNM 0.40 ± 0.07 vs control 0.45 ± 0.03 kcal/hr, p<0.01) and fifth week of treatment (dark period: AMP-DNM 0.52 ± 0.08 vs control 0.60 ± 0.05 kcal/hr, p=0.02; light period: AMP-DNM 0.41 ± 0.08 vs control 0.54 ± 0.05 kcal/hr, p<0.01, Table 1). Total animal activity did not differ between the treatment groups at any point during the whole experiment (data not shown).

Exposure to AMP-DNM decreases body weight gain

Lower body weight gain in the treated animals was noted during the first two weeks (gain as percentage of initial body weight: AMP-DNM 5.0 ± 5.3 vs control 14.1 ± 1.6%, p=0.00) and the second two weeks of treatment (AMP-DNM 7.1 ± 6.8 vs control 24.2 ± 4.0%, p=0.00). This had not yet resulted in a significantly lower body weight at the beginning of the third week of treatment, probably due to a slightly higher body weight in the treated group at the start of the experiment (Table 2). At the beginning of the fifth week and at the end of the experiment (day 37: AMP-DNM 46.2 vs control 51.3 gram, p=0.01) body weight was significantly lower in the treated animals.

Core body temperature did not differ significantly between both groups after 48 hours of treatment, but at the end of both the third and fifth week of treatment, it tended to be lower in the AMP-DNM treated animals (Table 2).

Table 2 Body weight, body weight gain and temperature during AMP-DNM treatment, values are presented as mean ± SD for eight mice in each group. Body weight gain and percentage body weight gain compared to initial body weight at the start of the experiment. * p<0.05 in AMP-DNm treated animals compared to controls.

	Week 1		Week 3		Week 5	
	AMP-DNM	control	AMP-DNM	control	AMP-DNM	control
Weight (g)	41.0 ± 2.4	39.9 ± 2.1	43.5 ± 2.8	45.5 ± 2.3	44.3 ± 3.3	49.5 ± 3.0
Weight gain (g)	-	-	2.1 ± 2.3	5.6 ± 0.6	3.0 ± 3.1	9.7 ± 2.3
Weight gain (%)	-	-	5.0 ± 5.3	14.1 ± 1.6	7.1 ± 6.8	24.2 ± 4.0
Temperature (C)	33.8 ± 3.8	36.8 ± 1.6	36.0 ± 0.5	37.1 ± 0.7	32.5 ± 3.7	36.5 ± 0.8

Treatment with AMP-DNM results in upregulation in beta-oxidation genes in liver and reduction hepatic fat content

Given the observation of increased fat oxidation rates in AMP-DNM treated ob/ob mice, we decided to study the expression of genes involved in fatty acid oxidation (CPT-1a, LCAD, PDK-4) in liver and muscle and the effects of treatment on liver lipid content. Mice were sacrificed after five weeks of treatment with AMP-DNM. We found increased hepatic expression of carnitine palmitoyl transferase-1a (CPT-1a, AMP-DNM 0.36±0.13 vs control 0.12±0.06, $p<0.01$), which is in line with the increased fatty acids oxidation rates. Expression of LCAD and PDK4 in liver was not significantly affected (data not shown).

Expression of the examined fatty acid oxidation genes in muscle was not changed after five weeks of AMP-DNM treatment (CPT-1a; AMP-DNM 0.15±0.03 vs control 0.23±0.10 p=0.09, LCAD; AMP-DNM 2.4±0.80 vs control 3.1±1.6 p=0.37, PDK4; AMP-DNM 4.5±2.3 vs control 3.3±0.9 p=0.19). As described previously (1;253) AMP-DNM lowered glucosylceramide in liver (AMP-DNM 0.11±0.02 vs control 0.27±0.06 nmol/mg protein, p=0.00), without altering ceramide levels (data not shown). AMP-DNM decreased liver triglyceride content by ~66 % (AMP-DNM 208±64 vs control 614±91 nmol/mg protein, p=0.00), most likely as a result of both the earlier observed decreased lipogenesis (17) and the here observed increased fat oxidation.

Four hour exposure to AMP-DNM does not change metabolic parameters

A switch from carbohydrate to fat oxidation may be explained by limitation of the availability of glucose for oxidation. To study this possibility, groups of six ob/ob mice received AMP-DNM or vehicle by gavage and were sacrificed after four hours. This short term exposure to AMP-DNM did not change plasma levels of

glucose (AMP-DNM 12.0 ± 2.3 mmol/l vs control 15.0 ± 3.4 mmol/l), insulin (AMP-DNM 15.5 ± 8.6 ng/ml vs control 9.2 ± 1.9 ng/ml), FFAs (AMP-DNM 0.60 ± 0.20 vs control 0.49 ± 0.22 mmol/l), triglycerides (AMP-DNM 0.83 ± 0.12 mmol/l vs. control 0.79 ± 0.12 mmol/l) nor of the intermediate metabolites pyruvate (AMP-DNM 109 ± 26 μmol/l vs control 130 ± 40 μmol/l), lactate (AMP-DNM 1.73 ± 0.75 mmol/l vs control 1.38 ± 0.50 mmol/l), and the ketone β-hydroxybutyrate (AMP-DNM 66 ± 33 μmol/l vs control 54 ± 51 μmol/l). A decrease in substrate availability is therefore unlikely to be the cause of acute effects of AMP-DNM on substrate oxidation patterns.

Table 3 Metabolic blood/plasma parameters during AMP-DNM treatment. Values are presented as mean ± SD for eight mice in each group. * p<0.05 in AMP-DNm treated animals compared to controls.

	Week 1		Week 3		Week 5	
	AMP-DNM	control	AMP-DNM	control	AMP-DNM	control
Glucose (mmol/l)	13.9 ± 5.2	19.5 ± 8.6	6.8 ± 1.2	10.8 ± 3.7*	6.5 ± 2.0	12.6 ± 5.1*
Insulin (ng/ml)	-	-	-	-	7.8 ± 2.6	9.7 ± 3.4
HbA1C (%)	-	-	-	-	4.3 ± 0.3	6.1 ± 0.7*
FFA (mmol/l)	-	-	-	-	1.2 ± 0.2	0.66 ± 0.10*
TG (mmol/l)	-	-	-	-	0.85 ± 0.22	0.62 ± 0.11*

Treatment with AMP-DNM results in increased levels of the regulatory protein peptide YY

Given the decrease in food intake and increase in fat oxidation we speculated that AMP-DNM treatment increases plasma levels of peptide YY (PYY). This short protein in released by L cells in the intestine in response to food intake and decreases appetite (17) and increases fatty acid oxidation (90). We measured plasma PYY levels in the five weeks AMP-DNM treated ob/ob mice, which were elevated by approximately 50% compared to control animals (AMP-DNM 0.65±0.10 vs control 0.43±0.05 ng/ml, p=0.00).

Discussion

Previous studies on the effect of AMP-DNM treatment in ob/ob mice showed marked improvement of insulin resistance, hepatic steatosis and inflammation (1;17;253). Here, we assessed the effect of AMP-DNM on substrate oxidation patterns and energy homeostasis. In the current study we show that treatment of ob/ob mice with AMP-DNM rapidly reduces food intake, increases fat oxidation and lowers carbohydrate oxidation. This change in substrate oxidation pattern did not result from reduced plasma glucose availability or increased plasma FFA

availability, since AMP-DNM did not affect plasma glucose, FFA or insulin levels after four or 36 hours of treatment.

These observations, in addition to our previous observation regarding the insulin sensitizing effects of AMP-DNM, suggest that AMP-DNM and related compounds may be relevant therapeutical approaches to improve the pathophysiology of obesity and insulin resistance.

It remains to be clarified how the reduction in food intake and the changes in substrate oxidation patterns are related. A reduction in body weight, as a result of a low caloric diet, is associated with an increase in fat oxidation in the fasted state in some studies, though others report no effect of weight loss on fatty acid oxidation (47). In the current study, the altered substrate oxidation pattern clearly preceded the reduction in body weight. This points towards effects of AMP-DMN on substrate oxidation patterns independent of changes in body weight.

AMP-DNM treatment increased fat oxidation whole body fat oxidation, which was associated with increased hepatic expression of genes involved in fat oxidation. In accordance with increased fat oxidation rates, AMP-DNM reduced hepatic steatosis in ob/ob mice. This inverse relationship between the rate of fat oxidation and the hepatic accumulation of triglycerides is also supported by other studies. For instance, tetradecylthioacetic acid increases β-oxidation by the induction of increased CPT-1 activity, which protects not only against diet-induced obesity and insulin resistance, but also lowers liver triglyceride content (154). Conversely, methyl-palmoxirate acutely inhibits beta oxidation by inhibition of CPT-1 activity, which results in increased liver triglyceride content even within 6 hours (64). Therefore, the stimulation of the expression of CPT-1 by AMP-DNM is in line with the reduction in hepatic triglyceride stores.

Four hour treatment with AMP-DNM did not change blood glucose, FFA and intermediate substrate concentrations nor did it change expression of genes involved in fatty acid oxidation in liver and muscle (data not shown in the article). Thus, the decrease in food intake and the increase in fatty acid oxidation are not secondary to peripheral metabolic effects of AMP-DNM. This suggests that there may be a direct effect of the compound on the regulation of satiety signals and substrate oxidation patterns. In the current study, we found increased concentrations of plasma peptide YY (PYY) in AMP-DNM treated animals. This peptide is released by L cells in the intestine in response to food intake, and has been shown to reduce appetite and slow gastric emptying, most likely due to stimulation of the Y2 receptor in the hypothalamus (12;125). Peripheral acute and chronic PYY administration has been demonstrated to stimulate fatty acid oxidation (251) and to reduce body weight rodent models of diet-induced obesity (259). Thus, the increase in PYY release from the gut in response to AMP-DNM

might explain, at least in part, the observed effects of AMP-DNM treatment. To further strengthen this observation, future studies are needed measuring PYY and other intestinal hormones at different time points after AMP-DNM treatment, in combination with food administrations. This should be combined with measurements of food intake and substrate oxidations patterns to establish the role of PYY in the effect of AMP-DNM treatment with more certainty.

In conclusion, treatment with AMP-DNM rapidly increases fat oxidation and decreases food intake. This is associated with an increase in PYY release from the L-cells in the gut. In addition to the insulin sensitizing capacities of AMP-DNM, the ability of this drug to increase fat oxidation and thereby reduce body fat mass gain and ectopic fat accumulation makes it an attractive candidate for the treatment of obesity and its metabolic complications