TETRA-O-METHYL NORDIHYDROGUAIARETIC ACID REDUCES WEIGHT GAIN IN MICE

by

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ABSTRACT

Tetra-O-methyl nordihydroguaiaretic acid (M4N) is a semi-synthetic derivative of the creosote bush molecule nordihydroguaiaretic acid (NDGA). NDGA alters the function and/or expression of components in the fat metabolic pathway, such as acetyl-CoA, malonyl-CoA, fatty acid synthase, and carnitine palmitoyltransferase-1. A benefit to using M4N over NDGA, however, is that M4N has reduced acute toxicity levels in vivo. We believed M4N was capable of altering the functions of these components similar to NDGA, and therefore would cause reduced body weight in mice fed M4N. The purpose of this study was to observe the effects of M4N on food consumption and weight accumulation in mice fed a high-fat diet. Results from this experiment showed that although mice with a high-fat diet (HF) and high-fat diet containing M4N (HFD) consumed nearly the same amount of food, HFs gained significantly more weight (31%) than HFDs (14%). The retroperitoneal and subcutaneous fat pads of HFs also appeared to be enlarged in comparison to HFDs. Taken together, this data suggests that similar to NDGA, M4N has the ability to alter fat metabolism and reduce weight gain in mice. Understanding mechanisms that allow for the control of fat metabolism has become of increased importance, as the rates of obesity have increased globally.

Ru Chih Huang, Ph.D. David Mold, M.D. Ph.D. Robert Horner, Ph.D.
ACKNOWLEDGEMENTS

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In addition, I would like to thank the Biology department of Johns Hopkins University. Special recognition goes to Dr. Robert Horner and Dr. Kathryn Tifft for their advising and mentorship during my time in the Masters program.
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INTRODUCTION

Obesity is a public health issue that is affecting populations on a global scale. Obesity is defined as someone who has a body mass index (BMI) greater than or equal to 30, while someone who is overweight has a BMI greater than or equal to 25. According to the World Health Organization, obesity has nearly doubled worldwide since 1980, with at least 2.8 million people dying each year as a result of being overweight or obese. In the United States alone, two-thirds of adults are either overweight or obese (1), which contributes to some of the leading causes of preventable death such as heart disease, stroke, type-2 diabetes, and certain forms of cancer (2). The effects of obesity are also manifested in metabolic syndrome.

At the core of obesity is an imbalance between energy intake and energy expenditure, which results in either increased fatty acid synthesis or fatty acid oxidation. Fatty acid synthase (FAS) catalyzes the reductive synthesis of long-chain fatty acids from acetyl-coenzyme A (acetyl-CoA) and malonyl-coenzyme A (malonyl-CoA) (3). Decreased FAS activity has been shown to decrease fat accumulation in peripheral tissues (4, 5). Carnitine palmitoyltransferase-1 (CPT-1) has been implicated in fatty acid oxidation as the rate-limiting step for the entry of fatty acids into the mitochondria (6). Malonyl-CoA is a competitive inhibitor of CPT-1, and functions to reduce the degradation of fatty acids when energy levels are excessive (6, 7). Therefore, directed treatment for components in the fat metabolic pathway could provide a way to target obesity.

Nordihydroguaiaretic acid (NDGA) is a compound that comes from the creosote bush Larrea tridentata of North America. NDGA serves an array of functions, such as
inhibiting lipoxygenase activity, inhibiting viral replication, and suppressing cancer cell growth (8). Tetra-o-methyl nordihydroguaiaretic acid (M4N) is a semi-synthetic molecule derived from NDGA. M4N has been previously studied for its anti-cancer effects, as it reduced Cdc2 RNA levels, protein levels, and enzymatic activity in solid tumors and induced apoptosis in human xenograft tumors (9-12).

Although both NDGA and M4N have been studied for their molecular mechanisms and clinical applications, a key difference between NDGA and M4N, however, is M4N’s reduced toxicity levels in vivo. The lethal dose of NDGA required to kill 50% of test animals (LD$_{50}$) via intraperitoneal injection was found to be 75mg/kg, while M4N was still tolerated at 1000mg/kg (13). The increased dosage tolerance of M4N makes it a more pragmatic molecule to investigate.

NDGA was also shown to affect different components involved in fat metabolism. NDGA was found to compete for FAS with the substrates acetyl-CoA and malonyl-CoA (14), all of which are involved in fat synthesis. NDGA was also found to inhibit FAS and upregulate CPT-1 (14, 15), giving promise to M4N’s ability to control fatty acid metabolism by working in a manner similar to NDGA. In addition to M4N’s potential as a source for obesity treatment, M4N has the ability to reduce the production of TNF-$\alpha$ (16), which is secreted from macrophages that are recruited to fatty adipose tissue. Because of NDGA’s background, we hypothesized that M4N might have applications as a metabolic regulator.

Metabolic syndrome is evidenced through a proinflammatory state, abdominally distributed weight, mild dyslipidemia, and hypertension, with the subsequent development of obesity, type 2 diabetes, and cardiovascular disease (17). Metabolic
syndrome combines disturbances in both glucose and insulin metabolism. Understanding M4N’s impact on weight and potential to function during fatty acid metabolism may provide an alternative method to combat the obesity epidemic.

MATERIALS/METHODS

Animals

C57BL/6J mice were used to model diet-induced obesity because their induced hepatic steatosis and hepatic insulin resistance are pathophysiologically similar to the human disease (18). All mice were managed under the guidelines of the International Review Board, as well as the Johns Hopkins University Animal Care and Use Committee. The housing and dietary methods used were similar to those performed by Murase et al (19). The experiment started with 15 C57BL/6J male mice (Jackson Laboratories) aged eight weeks old. Mice were kept under a 12-hour light/dark cycle in 23°C conditions, and were split into 3 groups: low-fat (LF), high-fat (HF), and high-fat containing the M4N drug (HFD). Both LF and HF mice were used as a control: HFs served as the control against M4N, and LFs served as a dietary control. Each group had a total of five mice, and each mouse had its own cage in order to track the amount of food that an individual mouse consumed. The experiment lasted a total of eight weeks.

Mouse HFD-4 was approximately half the weight (13.5 g) of all other mice, and subsequently died the second week of the experiment. Data from HFD-4 has been excluded from this analysis, leaving a total of 14 mice, with four mice in the HFD group.
**Diets**

The three diets consisted of the following ingredients:

a) *Low fat*: 120g basal mix, 40ml water, and 5ml corn oil

b) *High fat*: 120g basal mix, 5ml water, and 45ml corn oil

c) *High fat with M4N*: 120g basal mix, 5ml water, and 45ml corn oil with 25mg/ml M4N (6.83mg M4N per gram of food).

The basal mix (Harlan Laboratories) was composed of casein, L-cystine, cornstarch, maltodextrin, sucrose, cellulose, mineral mix, vitamin mix, choline bitartrate, and a TBHQ antioxidant. The nutritional composition of the basal mix (by weight) was 64.6% carbohydrate, 19.0% protein, and 0.2% fat.

Food was made periodically in bulk per diet, according to the need of each group. Mice were given *ad libitum* access to food and water. Unused food was stored at 4°C.

**Food Consumption and Weight Measurements**

Food consumption was measured by the total grams of food given, minus the total grams of food left in the cages during each check-in date. Measurements were taken three times a week. The HF and HFD food pellets were moister and would break easier compared to the LF diets. Because of the food pellet consistency, HF and HFD cages were not cleaned as frequently as LF cages in order to accurately account for all food on the measurement dates. HF and HFD fecal collection was also taken for possible analysis.
Mice were weighed once a week, and around the same time of day on each weigh-in date.

**Fat and Organ Collection**

On the final day of the experiment, at age 16 weeks, HF and HFD mice were sacrificed and dissected in order to retrieve the fat deposited at specific sites of the body. LF mice were excluded from fat collection. Fat pads were used as an indicator of fat metabolism, and were collected from the retroperitoneal, mesenteric, subcutaneous, and epididymal regions of the body. Fat pads were preserved using liquid nitrogen.

Blood and small liver segments were stored separately for future analysis. The brain, liver, kidneys, lungs, gastro-intestines, and pancreas were also removed and preserved using liquid nitrogen. These organs were stored by organ group, and pooled by either HF or HFD.

**Thin Layer Chromatography (TLC)**

TLC was performed in order to see if visible levels of M4N could be detected through an extraction process. A sample of brain, liver, kidney, lung, gastro-intestines, or pancreas was weighed, ground to powder, and dried down. Samples were then extracted in a methanol:acetonitrile:acetone (MeOH:ACN:Acetone;1:1:1) solvent using a volume five times the original weight of the tissue, and this mixture was incubated at room temperature overnight. The mixture was then centrifuged at 8,000 rpm for 30 minutes at 0°C, and the supernatant was saved for TLC. The pellet was then re-extracted using the
same volume of MeOH:ACN:Acetone, incubated at room temperature overnight, and centrifuged at 8,000 for 30 minutes at 0°C. The supernatant was again saved for TLC.

TLC was run using a hexane:ethyl acetate (3:1) eluent. Once dry, silicon plates were sprayed with a 5% sulfuric acid in ethanol solution, and then heated at 300°F for visualization. HF and HFD extracted tissues were run alongside 300ng, 150ng, 75ng, and 37.5ng M4N standards. M4N standards were created by first dissolving 4mg of M4N in 10ml of MeOH:ACN:Acetone (1:1:1), and then making serial dilutions using MeOH:ACN:Acetone (1:1:1).

**High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)**

HPLC-MS/MS was performed to further identify and quantify M4N levels. A sample of brain, liver, kidney, lung, gastro-intestines, pancreas, or fat\(^1\) was weighed, ground to powder, and dried down. Samples were then extracted using 5ml of a methanol:acetonitrile:acetone (MeOH:ACN:Acetone; 1:1:1) solvent. The solution was mixed and kept at -20°C for 20 minutes. Samples were then centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatant was removed and centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatant was again removed, and the sample was dried at room temperature. 500µl of ACN was added to the recovered sample, and then 10µl of this solution was used for HPLC-MS/MS. The following dilutions had to occur in order to perform analysis: gastro-intestines 100x, kidneys 10x, lungs 10x, HFD-2 fat pad 100x, and HFD-5 fat pad 10x.

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\(^1\) Fat was grouped by mouse, and not grouped by the location of the fat in the body.
For HPLC conditions, the column was a Phenomenex Luna 3µl C18(2) 150 x 2.0mm, with an injection volume of 10µl. The flow rate was 0.2mL/min and the MS run time was 70 minutes. The solvent was composed of varying ratios of 0.1% formic acid in water (FA_H₂O) and 0.1% formic acid in methanol (FA_MeOH). The initial solvent composition was 10% FA_MeOH at 0 minutes, and then was increased linearly to 100% FA_MeOH at 60 minutes. The solvent was kept at 100% FA_MeOH from 60 to 70 minutes.²

RESULTS

Mice were split into three groups to observe differences that result from consuming a low-fat diet, high-fat diet, and high-fat diet containing M4N, which lasted for a period of eight weeks.

Body Weights

The final average weights of each group showed that LFs gained the most weight, followed by HFs, and then HFDs (Figure 1A). While LFs and HFs had a steady weight increase for the duration of the eight-week period, HFDs averaged no net weight gain from weeks 0-2. HFDs slowly gained weight thereafter. By the end of the experiment, LFs had a final average weight of 30.7±1.9g with a gain of 8.7±2.5g. This represented a 40% weight increase. HFs had a final average weight of 29.0±2.0g with a gain of

² HPLC-MS/MS work was performed by our collaborator, Dr. Yu-Chuan Liang, at the Agricultural Biotechnology Research Center (ABRC), Academia Sinica, Taipei, Taiwan.
6.8±2.6g, representing a 31% increase. HFDs had a final average weight of 26.0±1.4g with a gain of 3.3±0.5g, representing a 14% increase.

**Food Consumption**

LF mice consumed the greatest amount of food, followed by HFs, and then HFDs (Figure 1B). However, HFDs did not eat less food than HFs for the entirety of the experiment. From day 9 to day 33, HFDs actually consumed slightly more food than HFs, while HFs ate more food than HFDs from day 40 until the end of the experiment (Figure 1B). This indicates that the lack of excessive weight gained by HFDs was not due to a reduced appetite, but rather points to a more mechanistic method affecting weight accumulation.

LFs consistently consumed the most amount of food overall, as they averaged 4.2±0.2g/day for a total of 225.1g over the eight-week period. HFs averaged 3.4±0.2g/day for a total of 183.9g of food. HFDs averaged 3.3±0.3g/day for a total of 178.1g of food. The average HFD M4N intake was 22.5±2.2mg/day for a total of 1216 mg.

**Tissue and Fat Composition**

Fat pads from the retroperitoneal, mesenteric, subcutaneous, and epididymal regions of the body were compared upon removal (Supplementary Figures 1 and 2). There appeared to be a reduction in the amount of retroperitoneal fat in HFD mice. This was most evident in HFD-3 and HFD-5 (Figure 2). Reduction in subcutaneous fat was
also observable in HFD-1, HFD-3, and HFD-5 (Figure 2). There did not appear to be a significant difference between the mesenteric and epididymal fat between HFs and HFDs.

HPLC-MS/MS detected M4N in the gastro-intestines, kidneys, brain, lungs, pancreas, liver, and the fat pads from HFD-1, HFD-2, HFD-3, and HFD-5 (Table 1, Supplementary Figures 3-12). Of the non-fat samples, the gastro-intestines had the highest concentration of M4N, while the liver had the lowest concentration of M4N.

**DISCUSSION**

The ability to regulate fat metabolism is one way to target obesity, and M4N demonstrated its potential to manage weight based on the activity of NDGA. As such, the aim of this experiment was to assess the effects of M4N on fat accumulation and measure how much M4N reaches different tissues by oral feeding of the drug. Here, we show evidence that M4N reduced the amount of weight gained in mice and that M4N is also detectable in various tissue samples.

**Diet and Weight Gain**

A major focus of this experiment was to observe how weight could be affected by M4N. Our results show that there is a negative correlation between weight gain and the use of M4N. The average final weight of the HFDs was less than that of HFs. The data shows that as HF and HFD mice aged, the disparity between their weights grew larger (Figure 1A). One interesting observation to note is the food consumption rates
between HFs and HFDs. Although HFs and HFDs ate nearly the same amount of food for the duration of the experiment, HFs were steadily gaining weight while the weight of HFDs initially remained constant, but then slowly increased (Figure 1). Because both groups of mice ate a similar quantity of food, we hypothesized that M4N was acting at the level of fat metabolism in order to reduce weight gain. If M4N was working to curb appetite, there should have been a difference between the amounts of food that each group consumed, therefore resulting in the differences seen between group body weights. On average, JAX® C57BL/6J male mice weigh 24.0 ± 1.5g at eight weeks old when fed a diet containing 6% fat. By age 16 weeks, the male mice’s average weight increases to 30.1 ± 2.1g (20), representing a 25% weight gain. HF mice in this study had a 31% weight increase, while HFDs had an increase of 14%. This information suggests the weight gained by HFD mice was a result of developmental requirements. Although weight gain is necessary for development, excessive fat accumulation can lead to detrimental health outcomes such as hypertension, type-2 diabetes, and coronary heart disease (2). Therefore, M4N’s potential to reduce weight gain may prove beneficial to health.

Because it appeared as though the retroperitoneal and subcutaneous regions were smaller in HFDs compared to HFs, it may be useful to know why these areas of the body seemed to be more affected by M4N. Subcutaneous fat is often associated with central abdominal adiposity and has a strong connection with insulin resistance (21). Since all fat pads did not appear to be reduced to an equal degree, this difference could reveal certain pathways by which M4N acts.
One important observation to note was the heightened food intake and weight gain among LFs (Figure 1B). HFs and HFDs were fed a high-fat diet so that the development of metabolic syndrome could resemble the pathogenesis of the disease found in humans (17). Even if LFs consumed more food, the initial thought was that they might not gain as much weight due to the composition of their diet. This proved to be false since LFs remained the largest group. The difference in food intake between the low-fat and high-fat groups could be due to their preference toward the food taste or texture. Since mice had *ad libitum* access to food and water, there was no restriction on the amount of food they were allowed to consume. However, because LFs ate 41.2g more food than HFs, we cannot exclude the possibility that LFs would have had a comparable final weight if both groups had similar consumption rates. In fact, it is more telling to look at the ratio of the amount of weight gained to the amount of food eaten. LFs had a ratio of 3.9%, HFs had a ratio of 3.7%, and HFDs had a ratio of 1.9%. Nevertheless, because there was a greater similarity between the food make-up of HFs and HFDs, we focused on differences found between HFs and HFDs.

Our results show the gastro-intestines had the highest M4N concentration, while the liver had the lowest concentration of M4N (Table 1). The gastrointestinal tract extends from the mouth to the anus, and the small intestine is the location where an orally administered drug is most absorbed (22). Excluding the fat pads, the gastrointestinal sample was also one of the hardest samples to dry down due to its increased oil content. Since M4N is a lipid soluble molecule, the higher concentration of M4N in the gastro-intestines could be owing to the retention of M4N in the gastrointestinal tract. In addition, fecal pellets within the gastro-intestines could contribute to the M4N
concentration, in the event that M4N was being excreted from the body. In the case of the M4N concentration found in the liver, the liver is the principal site for drug metabolism (23). Metabolism of a drug suggests the drug will no longer be detectable in its original form. Due to the vital metabolizing function of the liver, it is not surprising that levels of M4N detected in the liver were lower in comparison to the other tissue samples. This study is also the first instance that shows M4N can be identified in brain tissue (Supplementary Figure 5). It was previously thought that M4N may not be detectable in the brain due to the blood-brain barrier, but here we show evidence that M4N is measurable in the brain.

Another notable observation among the three groups is that while all mice behaved similarly for the duration of the experiment, HFs and HFDs had a marked presence of greasy-looking fur by the second week. The area around their necks was the most affected region, which cleared up by the sixth week. This observation could be attributed to the higher oil content of the HF and HFD diets, as this type of appearance was not exhibited by LFs.

Possible Mechanisms by Which M4N May Act

The mechanism by which M4N acts is unknown, but evidence offers possibilities based on its parent molecule, NDGA. FAS catalyzes the synthesis of long-chain fatty acids, and decreased FAS activity causes a decrease in fat accumulation in peripheral tissues (3-5). A previous study found that NDGA inhibited FAS by acting competitively with acetyl-CoA and noncompetitively with malonyl-CoA (14). Acetyl-CoA is converted into malonyl-CoA by acetyl-CoA carboxylase (ACC), and malonyl-CoA is a
direct substrate of FAS (3). Since NDGA reduces FAS activity by way of acetyl-CoA and malonyl-CoA, it is possible that M4N behaves in a similar manner. The use of M4N, which is essentially nontoxic, would be more beneficial for use in clinic to alter different components involved in the fat metabolic pathway.

Another molecule implicated in fat metabolism is CPT-1, since fatty acid oxidation in the mitochondria is regulated by the presence of CPT-1. When CPT-1 is available, acyl-conenzyme A is converted into acylcarnitine, thereby allowing for the progression of β-oxidation. An inhibitor of CPT-1 is malonyl-CoA, which reduces the breakdown of fatty acids when an abundance of energy is available (6,7). Moreover, AMP-activated protein kinase (AMPK) inhibits ACC while upregulating CPT-1 (24). If ACC is inhibited, the conversion from acetyl-CoA to malonyl-CoA is reduced, thereby limiting the activity of FAS. In addition to reducing FAS activity, NDGA was found to downregulate ACC, while upregulating the expression of CPT-1 by supporting the activation of AMPK (15). If M4N is capable of both upregulating CPT-1 and downregulating FAS, this relationship would further promote the progression of fatty acids to undergo β-oxidation.

**Long-Term Effects of M4N**

M4N has great potential to be studied in relation to weight management, and a longer-term study could better test the effects of M4N on weight gain, weight loss and food consumption. For example, because HFD food consumption appeared to slightly taper off towards the end of the experiment (Figure 1B), it would be interesting to see if M4N has the ability to diminish appetite after an extended period of time. In addition, a
future study may want to start with mice that are overweight and see how M4N affects the mice at that stage of life. This method would better mimic the conditions of obesity found among humans and discern if the effects of M4N can cause weight loss rather than just reduce the amount of weight gained.

**M4N May Help Regulate Blood Glucose Levels**

Obesity is further complicated by the altered production of proinflammatory molecules, such as tumor-necrosis factor-α (TNF-α), which are produced by macrophages that are recruited to adipocytes (25,26). Inflammation that arises as a result of enlarged fat cells has also been implicated in insulin resistance (27) and TNF-α is inhibited by M4N (16). So, in addition to weight management, M4N may have the ability to affect total body insulin levels. The higher the concentration of free fatty acids in the body, the less sensitive the body becomes to insulin, resulting in reduced glucose uptake (26,28). Macrophages that accumulate in adipose tissue have been associated with obesity and contribute to the major source of TNF-α found in these tissues (25). As adipocytes grow, they secrete monocyte chemoattractant protein-1 (MCP-1), which recruits macrophages and elevates macrophage infiltration (29). It was previously found that when comparing macrophage content in the adipose tissue of lean and obese individuals, lean individuals had a macrophage content up to 10%, while obese individuals had a macrophage content up to 50% (26). In essence, the recruitment of macrophages to adipose tissues induces an inflammatory response. As M4N has been shown to reduce levels of TNF-α (16), a reduction of TNF-α via M4N may help alleviate the insulin-resistant inflammatory response, and help regulate blood glucose levels.
In all, it will be important to elucidate the mechanism by which M4N acts in order to understand how it affects fat metabolism and fat accumulation. As this study has shown M4N’s ability to reduce the total amount of weight gained in mice that consume a high-fat diet, the future of M4N research looks promising, as it can offer another approach to combat the obesity epidemic.
Figure 1. Average body weight and food intake of each diet group. (A) Body weight of each group over a period of eight weeks. At day zero, mice were eight weeks old. LFs started at 22.0g and gained 8.7g; HF's started at 22.2g and gained 6.8g; and HFD's started at 22.8g and gained 3.3g. (B) LFs consumed the most amount of food while HF's and HFD's consumed similar amounts of food.
Figure 2. M4N reduced retroperitoneal and subcutaneous fat pads in high fat + M4N (HFD) mice. Fat was removed from the retroperitoneal (A), mesenteric (B), subcutaneous (C), and epididymal (D) regions of the body. Arrows point to the retroperitoneal region, while chevrons point to the subcutaneous region. The HF and HFD number corresponds to the specific mouse observed.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total M₄N (μg)</th>
<th>M₄N / wet weight (μg/g)</th>
<th>M₄N conc. (μM)</th>
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</thead>
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<tr>
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<td>14.86</td>
<td>41.50</td>
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<tr>
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<td>0.99</td>
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<td>Lung</td>
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<td>Pancreas</td>
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<tr>
<td>HFD-5 fat pads</td>
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</table>

Table 1. **Concentrations of M₄N are detectable in tissues.** Tissue samples were dissected, dried, and underwent HPLC/MS-MS to measure if concentrations of M₄N could be detected. M₄N concentrations were determined based on the amount of M₄N extracted from the dry tissue sample, and then correlated back to the wet weight of the original tissue sample. The standard curve for these calculations had a range of 0.1 ng/μL to 10 ng/μL. Data from HFD-4 has been excluded.
Supplementary Figure 1. High-fat (HF) fat pads. Fat was removed from the retroperitoneal (A), mesenteric (B), subcutaneous (C), and epididymal (D) regions of the body. Arrows point to the retroperitoneal region, while chevrons point to the subcutaneous region. The HF number corresponds to each mouse observed.
Supplementary Figure 2. High fat + M4N (HFD) fat pads. Fat was removed from the retroperitoneal (A), mesenteric (B), subcutaneous (C), and epididymal (D) regions of the body. Arrows point to the retroperitoneal region, while chevrons point to the subcutaneous region. The HFD number corresponds to each mouse observed. Data from HFD-4 has been excluded.
Supplementary Figure 3. M4N is detectable in the gastro-intestines. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 201,917,961 absorbance units and a retention time of 58.04 minutes. (B) The intensity peak at 358.95 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 4. M4N is detectable in the kidneys. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 20,220,389 absorbance units and a retention time of 58.04 minutes. (B) The intensity peak at 358.96 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 5. M4N is detectable in the brain. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 128,770,773 absorbance units and a retention time of 57.89 minutes. (B) The intensity peak at 358.97 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 6. M4N is detectable in the lungs. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 10,078,178 absorbance units and a retention time of 58.04 minutes. (B) The intensity peak at 358.96 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 7. **M4N is detectable in the pancreas.** Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 69,683,641 absorbance units and a retention time of 57.82 minutes. (B) The intensity peak at 358.96 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 8. M4N is detectable in the liver. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 236,059,423 absorbance units and a retention time of 57.85 minutes. (B) The intensity peak at 358.96 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 9. M4N is detectable in HFD-1 fat pads. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 50,327,752 absorbance units and a retention time of 57.78 minutes. (B) The intensity peak at 358.97 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 10. M4N is detectable in HFD-2 fat pads. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 16,764,962 absorbance units and a retention time of 57.89 minutes. (B) The intensity peak at 358.99 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 11. M4N is detectable in HFD-3 fat pads. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 102.903.429 absorbance units and a retention time of 57.77 minutes. (B) The intensity peak at 358.97 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
Supplementary Figure 12. M4N is detectable in HFD-5 fat pads. Tissue samples were dissected, dried, and underwent HPLC/MS-MS to identify the presence of M4N. (A) M4N peaked at an atomic absorption of 24,142,595 absorbance units and a retention time of 57.75 minutes. (B) The intensity peak at 358.98 daltons confirmed the presence of M4N. Red boxes indicate peaks corresponding to M4N.
References


