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The Combination of S-adenosylmethionine and Dilinoleoylphosphatidylcholine Attenuates Non-alcoholic Steatohepatitis Produced in Rats by a High-Fat Diet

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Abstract

In the pathogenesis of non-alcoholic steatohepatitis (NASH), oxidative stress resulting from free radicals generated by cytochrome P4502E1 (CYP2E1) plays a major role suggesting the importance of antioxidants. The objective of this study was to assess in a high-fat diet (HF) rat model the effects of the combination of s-adenosylmethionine (SAME) plus dilinoleoylphosphatidylcholine (DLPC) in the treatment of NASH. To test the hypothesis that these two antioxidants are beneficial in NASH, male Sprague-Dawley rats were fed five different diets for six weeks: control, HF diet and HF plus SAME and DLPC or their combination. As expected, the HF diet significantly increased hepatic triacylglycerols and CYP2E1 levels. However, only the combination diet opposed this effect, consistent with different actions of the two antioxidants. Next, 24 additional rats divided in two groups were fed the HF or the HF+SAME+DLPC diets for 3 weeks. Dietary intake was similar, but liver triacylglycerols dropped from 76.1 ± 6.8 to 49.4 ± 3.5 mg/g ($p=0.002$) and hepatic CYP2E1 mRNA decreased after treatment ($p=0.01$) with a trend for less CYP2E1 protein. This was accompanied by a 41% reduction of hepatic 4-hydroxynonenal (4-HNE) ($p=0.008$), reflecting control of oxidative stress. Furthermore, the hepatic inflammatory cytokine tumor necrosis factor- α (TNF- α) mRNA and TNF- α protein decreased ($p=0.05$ and $p=0.01$ respectively) with attenuation of $\alpha 1(I)$ procollagen mRNA and type I collagen levels ($p=0.01$ and $p=0.02$, respectively). We concluded that the combination SAME+DLPC might be beneficial in NASH by reducing oxidative stress and associated liver injury.

Keywords

Non-alcoholic steatohepatitis (NASH); Fat; s-adenosylmethionine (SAME); dilinoleoylphosphatidylcholine (DLPC); Rats

1. Introduction

The pathogenesis of non-alcoholic steatohepatitis (NASH) is multi-factorial with one major mechanism being that oxidative stress is generated by the induction of cytochrome P4502E1 (CYP2E1). With increased aging and weight-gain in our population, obesity and diabetes has

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become frequently associated with non-alcoholic fatty liver disease (NAFLD). Whereas NAFLD is usually benign, NASH is recognized as a precursor to “cryptogenic” cirrhosis [1]. Nonalcoholic fatty liver (NAFL) and NASH combined, are now the most common liver diseases in the USA [2]. Lipid peroxidation is considered to be the trigger factor responsible for the transition from simple fat accumulation to eventually more progressive steatohepatitis or NASH [3]. In fact, a number of studies have now clearly established that the oxidative stress associated with liver disease (and found to be the main mechanism for pathogenesis) is associated with, and exacerbated by, a striking depletion of s-adenosylmethionine (SAME) and other supernutrients, such as phosphatidylcholines (PC). One of the key functions of SAME is to act as a methyl donor in the methylation of phosphatidylethanolamine to PC [4]. However, chronic liver disease, such as the one induced by chronic ethanol consumption, is associated with a decrease in the activity of phosphatidylethanolamine methyl transferase (PEMT). Decreased activity of PEMT (associated with alcoholic liver injury) depletes phosphatidylcholine in the membranes, further impairing the activity of PEMT and of other key membrane enzymes, such as those associated with oxidative phosphorylation. Administration of PC with polyenylphosphatidylcholine (PPC) or dilinoleoylphosphatidylcholine (DLPC), its main and highly bioavailable component restores the level of PC and breaks the vicious cycle.

In experiments thus far, PPC (or DLPC) and SAME have been administered separately to animals; although they were beneficial, the effects of each separately were incomplete. This finding is not surprising since the deficiencies in SAME and PC are interdependent [4]. SAME alone cannot be expected to be fully corrective since, in its function of methylation of phosphatidylethanolamine to phosphatidylcholine, it depends on the normal activity of the corresponding enzyme, namely PEMT, which is depressed by liver disease but can be reactivated by PPC [5]. Conversely, optimal activity of this enzyme requires ample supply of its cofactor SAME, which is depleted in alcoholic liver disease [6]. Several studies have shown the fundamental role that SAME plays in maintaining normal liver functions [7] and how much SAME deficiency predisposes the liver to injury [8] and chronic hepatic deficiency results in spontaneous development of steatohepatitis and hepatocellular carcinoma [9]. The logical conclusion would be to base therapy on the administration of SAME + DLPC. Thus, the approach in this experimental design assesses whether the administration of these two nutraceuticals together results, as hypothesized, in a significant synergistic effect.

A suitable animal model for NASH, expressing all responsible factors for the pathogenesis of NASH such as CYP2E1 induction, has been recently described by us [10]. Rats were fed a high-fat diet (HF), which generated the key complications of NASH. There is no consensus on the effectiveness of various proposed therapies, but *in vitro* studies have shown a remarkable decrease in oxidative stress due to the combination SAME + DLPC.

The rationale of the present study was to test the hypothesis that NASH could be attenuated by inhibitors of the oxidative stress and CYP2E1 induction. To that effect, we used two physiologic agents that inhibit CYP2E1's oxidative stress, namely SAME [11-13] and DLPC [14], both of which were recently found in *in vitro* experiments to act synergistically when combined [15;16]. The use of antioxidants and their combined action has been proposed for the treatment of various diseases [17]. This is the first study indicating the usefulness of this approach in the field of nutrition.

2. Methods and materials

2.1. Animals and Diets

In a preliminary experiment 25 male Sprague-Dawley rats purchased from Charles River Laboratories (Wilmington, MA) were divided in 5 groups and fed *ad libitum* 5 different diets

for 6 weeks. Group 1 received the nutritionally adequate control liquid diet containing 35% of fat [10], group 2 was given the NASH HF liquid diet with 71% of energy derived from fat, 18% from proteins, 11% from carbohydrate [10]; group 3 received the HF diet with SAME 400 mg/1000 calories; group 4 the HF diet with 1.5 gm/1000 calories of DLPC, a soybean extract purchased from Avanti Polar Lipids (Alabaster, AL) and which is the active component of PPC and group 5 the HF diet with SAME and DLPC combined in the amount described above. Subsequently, an additional 24 male Sprague-Dawley rats were divided in two groups and fed *ad libitum* only the HF diet with or without 400 mg/1000 calories of SAME and 1.5 gm/1000 calories of DLPC for 3 weeks. For all the experiments the basic diet (Table 1) was purchased from Dyets Inc. (Bethlehem, PA). The animals were killed in the fed state by exsanguination (under light pentobarbital anesthesia). The animal protocols and use of rats was approved by the James J. Peters VA Medical Center subcommittee on animal studies Institutional Animal Care and Use Committees (IACUCs), a program fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

2.2. Organelle Preparation

Livers were homogenized and centrifuged at $8700 \times g$ and 4°C for 10 min. The postmitochondrial supernatant was recentrifuged at $100,000 \times g$ at 4°C for 1 hr to separate microsomes from the cytosol [18]. Aliquots of the homogenates and the microsomes were frozen in liquid nitrogen and stored at -80°C .

2.3. CYP2E1 protein expression

Measurements of CYP2E1 were carried out by Western blots. Microsomal proteins were subjected to sodium dodecyl sulfate-polyacrylamide (SDS) 10% gel electrophoresis as previously described [19]. After transfer to a nitrocellulose membrane, CYP2E1 was revealed with rabbit polyclonal anti-hamster CYP2E1 IgG (produced in our laboratory). A known microsomal control was used as standard and for calculation of density. In some experiments, to assess equal protein loading, blots were stripped and incubated with anti-calregulin antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) a known marker for microsomal fraction CYP2E1. Bands were quantitated by densitometry using Evaluating Image Analysis Systems MCID (Imaging Research Inc., St. Catherines, Ontario, Canada).

2.4. Measurement of Lipids

Liver triacylglycerols were measured by reagents obtained from WAKO Chemicals USA, Inc. (Richmond, Virginia). A lipid calibrator was used for the standard curve and samples values calculation.

2.5. Measurement of Lipid Peroxidation

In the liver, oxidative stress was assessed by the concentration of 4-hydroxynonenal (4-HNE), which was measured by gas chromatography-mass spectrometry according to van Kuijk et al [20] with minor modifications [21]. Separation of O-pentafluorobenzyl oxime trimethylsilyl ether derivatives of 4-HNE will be performed on a DB 5 capillary column, 15 meter long, inner diameter 0.25 mm, film thickness 0.25 μm (J&W Scientific, Folsom, CA). The injection port temperature was 270°C ; the gas chromatograph oven temperature was programmed from 60° to 205°C at $25^{\circ}\text{C}/\text{min}$. 4-HNE was monitored at amu 152 while deuterated [$^2\text{H}_3$]-4-HNE was used as an internal standard, at amu 155. The syn-peak of 4-HNE was eluted 0.01 to 0.02 min before the peak of the internal standard. The filament emission current was 175 to 250 μA and results were expressed as % of control.

2.6. ELISA assays for Tumor necrosis factor α (TNF- α) and Collagen type I protein expression

Snap-frozen rat liver tissues was homogenized on ice in a sample buffer (50 mM Tris, pH 7.6, 0.25% Triton X-100, 0.15 M NaCl, 10 mM CaCl₂) containing serine- and thiolprotease inhibitors (PMSF 0.1 mM, leupeptin 10 μ M, pepstatin A 10 μ M, aprotinin 25 μ g/ml, iodoacetamide 0.1 mM). The homogenate was centrifuged at 4°C and 14,000g for 30 min to remove cell debris and protein aggregates. The protein concentration was measured with the BCA protein assay kit (Pierce, Rockland, IL). 50 μ l of the obtained supernatant was used to determine the concentration of TNF- α by ELISA using either the Quantikine Rat ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions or the ELISA method described by Moshage et al [22]. Data were expressed as pg/mg protein and ng/mg protein, respectively.

2.7. Extraction of mRNA and Northern Blots

Liver tissue (20-30 mg) collected under sterile conditions and quickly frozen in liquid nitrogen was subjected to total RNA isolation by phenol: chloroform extraction and further enriched using RNeasy mini columns (Qiagen) with digestion of RNase-free DNase (Qiagen). TNF- α mRNA was measured using the Quantikine mRNA Bas, Probes and calibrator kits (R&D Systems, Minneapolis, MN). α 1(I) procollagen mRNA expression was assessed by Northern Blots as published before [10]. Twelve μ g of RNA per sample were subjected to electrophoresis and transferred to a Genescreen membrane. The membranes were prehybridized and hybridized with β -actin, α 1(I) procollagen probes obtained from the American Type Culture Collection (Manassas, VA) and CYP2E1 from Oxford Biochemical Research Inc. (Oxford, MI). All probes were labeled with P³² 2'-deoxycytidine 5'-triphosphate using a random priming DNA labeling kit (Amersham, Arlington Heights, IL). The membranes were exposed to radiographic film (Kodak, Rochester, NY) and the intensity of the bands was quantified with the Evaluating Image Analysis Systems MCID (Imaging Research, St Catharines, Ontario, Canada). Data were expressed as a ratio of the densitometric units to β -actin.

2.8. Plasma assays

Measurement of triacylglycerol was carried out with the WAKO L Type TGH kit (Richmond, VA). Insulin and leptin concentrations were assessed by ELISA kits obtained from Crystal Chemical Inc. (Downers Grove, FL) and adiponectin by the ELISA kit from B-Bridge International (Sunnyvale, CA).

2.9. Morphology

Liver tissue was fixed in neutral-buffered formalin solution for no more than 24 hr, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. Sections were stained by haematoxylin and eosin, trichrome and Sirius red and then scored blindly for overall pathology.

2.10. Statistical Analysis

Data were analyzed by One Way Analysis of Variance (ANOVA) followed by the Student Newman-Keuls post hoc test and Student's t test for two groups comparison. All statistical analyses were performed with the INSTAT version 3 (Graph Pad 3 Software, San Diego, CA) (www.graphpad.com). For the ANOVA, the equality of variance was assessed by the Barlett's test. No outliers were eliminated. Results were expressed as means $\pm$ SEM. A p value of <0.05 was considered as statistically significant [23].

3. Results

In both experiments (3 and 6 weeks) there was no significant difference in weight gain among all groups (Tables 2,3). After six weeks of treatment, as expected, compared to control, with

the HF diet there was a significant fat accumulation. In this model, SAME or DLPC alone were not effective, while the combination of the two resulted in less steatosis as shown by the hepatic triacylglycerols levels (Fig. 1A). In addition, CYP2E1 significantly increased with the HF diet and remained unchanged in the HF supplemented with either SAME or DLPC, but was significantly reduced by the combination of the two (Fig. 1B). 4-HNE level was 15% higher with the HF diet but it was not significantly different from control and neither SAME nor DLPC or their combination affected the HF diet values (data not shown). The high body weight (Table 2) and liver fat accumulation may have affected this response.

Since, as published before [10] a NASH model can be obtained with only 3 weeks of treatment, accordingly, in another experiment, we compared only the HF diet to the one supplemented with SAME+DLPC. After 3 weeks of treatment the combination of SAME+DLPC significantly reduced histological hepatic steatosis (Figs. 2,3) and hepatic triacylglycerols levels (76.1 ± 6.8 vs. 49.4 ± 3.5 mg/g with SAME+DLPC treatment; $p=0.002$) (Fig. 2C). Hepatic CYP2E1 mRNA also significantly decreased after SAME+DLPC from 104.8 ± 15.5 to 53.6 ± 10.0 density units ($p=0.01$) as measured by Northern blots (Fig. 3), whereas there was only a trend for the decrease in CYP2E1 protein from 38.5 ± 4.9 to 26.3 ± 3.9 density units ($p=0.07$). A decrease of CYP2E1 would be expected to reduce products of the CYP2E1 induced oxidative stress and, indeed, one such product, namely hepatic 4-HNE, dropped by 41% compared to the HF diet alone ($p=0.008$) (Fig. 4). Furthermore, the inflammatory cytokine TNF- α decreased from 104.5 ± 6.3 to 81.7 ± 3.6 pg/mg protein ($p=0.05$) (Fig. 5A) and the TNF- α mRNA was reduced from 808 ± 26 to 675 ± 33 mol/mg protein ($p=0.01$) (Fig. 5B). Moreover, parameters related to fibrosis, such as $\alpha 1(I)$ procollagen mRNA and collagen, were diminished from 2595 ± 249 to 1904 ± 63 density units ($p=0.01$) (Fig. 6A) and from 677 ± 21 to 607 ± 19 pg/mg protein ($p=0.02$), respectively (Fig. 6B). However, there was only a trend for a decrease in plasma insulin, leptin and adiponectin (Table 4).

4. Discussion

This study demonstrates for the first time that in an experimental rat model of NASH [10], the combination of two physiologic antioxidants, namely SAME and DLPC, have a synergistic effect and significantly oppose liver injury generated by the excess of fat. Since the initial proposition by James and Day [1] for the “two hit” model of NASH, the new evolving view is that fat accumulation, inflammation, necrosis of hepatocytes, and cell death occur simultaneously and are responsible for a series of molecular events involving nuclear transcriptional factors leading to insulin resistance, mitochondrial dysfunction, CYP2E1 induction and lipid peroxidation [3]. These features were reproduced in animals fed a high fat liquid diet. After six weeks of strict controlled feeding, only the combination of SAME+DLPC was effective, whereas SAME and DLPC alone did not have a significant effect on the parameters measured. Other animal models of NASH have been studied as reviewed by Nanji [24]. Agent-stimulating glutathiones, such as SAME and 2(RS)-n-propylthiazolidine-4(R)-carboxylic acid, have been found to be hepatoprotective in rats fed a methionine-choline deficient (MCD) diet [25]. However, the combination of two innocuous compounds largely available and used by the general population, such as SAME and DLPC, has never been tested before in conjunction with a balanced liquid diet able to reproduce all the pathophysiological “hit” characteristics of human NASH. A high saturated fat diet administered to obese rats accelerates liver damage, thereby acting as a second “hit” mechanism [26]. Our study demonstrates that the combination of SAME + DLPC down-regulates CYP2E1. This effect is possibly attributed to DLPC, which has been shown before to be an effective inhibitor of CYP2E1 induced by chronic alcohol consumption [27]. SAME, by contrast, is a methyl-donor compound largely studied after chronic alcohol consumption [28;29] because of its capacity to restore in the liver the decreased SAME and glutathione levels [30], thereby improving mitochondrial functions [29].

However, the antioxidant effect illustrated by the reduction in 4-HNE in rats fed HF+DLPC +SAmE can be attributed to the combined action of the two compounds since both have the property of being powerful antioxidants: DLPC reduces lipid peroxidation, possibly through decreased CYP2E1 [19] while SAmE does so by restoring GSH and mitochondrial function. NASH is the result of multiple “hits,” and its pathogenesis includes impaired mitochondrial functions resulting in lipid peroxidation generated by the induction of CYP2E1. In fact, as recently reviewed [17], its treatment has been focused on the combined actions of antioxidants. It is evident that any therapeutic intervention should involve a reduction in the oxidative stress resulting from the CYP2E1 induction by the HF diet, one of the well established pathogenic mechanisms of NASH [31-33].

This *in vivo* study in rats confirms previous observations from *in vitro* experiments suggesting that the combination of SAmE + DLPC is very effective [15;16]. Indeed, in this study, the increase in 4-HNE, a marker of oxidative stress, was significantly attenuated by the antioxidants SAmE + DLPC. There was also a significant reduction in a number of key pathologic changes associated with NASH including not only the oxidative stress, but also the inflammatory cytokine TNF- α and the steatosis.

However, it is difficult to demonstrate the relative effect of one antioxidant versus the other. Yet, one may speculate that SAmE, by improving DNA methylation [34], is the key agent in restoring mitochondrial function and blocking collagen production [35]. We observed that some precursors of fibrosis, namely $\alpha 1$ (I)procollagen mRNA and type I collagen, are improved by the combination of SAmE+DLPC. Possibly because of a short treatment time, we were unable to show an effect on insulin resistance and the hormonal levels of leptin and adiponectin.

The results of the present study have obvious implications for possible prevention and/or treatment of NASH. Thus far, there is no consensus on whether any of the proposed therapies have been clearly effective. The pathophysiological role of antioxidant therapy in chronic liver disease becomes the main therapeutic option [36]. Given that one common cause of NASH is obesity, an ongoing debate exists regarding what constitutes the best diet to control obesity. Accordingly, it might be of interest to test the protective effect of SAmE + DLPC in patients with NASH, in view of the innocuity of these two compounds and their effectiveness in our rat model. In summary, we discovered in a rat model of NASH that the combination of SAmE + DLPC reduces the oxidative stress resulting from the CYP2E1 induction generated by an excess of lipids, thereby significantly attenuating key changes associated with NASH. Since SAmE and DLPC are innocuous compounds, clinical usage in NASH patients may now be considered.

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Abbreviation List

4-HNE	4-hydroxynonenal
CYP2E1	Cytochrome P4502E1
DLPC	Dilinoleoylphosphatidylcholine
HF	High-fat
MCD	Methionine-choline deficient
NAFLD	Non-alcoholic fatty liver disease
NAFL	Nonalcoholic fatty liver
NASH	Non-alcoholic steatohepatitis
PC	Phosphatidylcholines

PEMT	Phosphatidylethanolamine methyl transferase
PPC	Polyenylphosphatidylcholine
SAMe	S-adenosylmethionine
SDS	Sodium dodecyl sulfate-polyacrylamide
TNF-α	Tumor necrosis factor α

Figure 1. After six weeks of treatment, rats fed a HF diet supplemented with SAMe+DLPC showed significant effects

(A) Effect of SAMe + DLPC on hepatic triacylglycerols. The combination of SAMe+DLPC significantly decreased the liver triacylglycerols measured by a kit obtained from WAKO.

(B) The increase in CYP2E1 after a HF diet was also significantly reduced by SAMe+DLPC. Microsomal proteins (50 μ g) were subjected to sodium dodecyl sulfate-polyacrylamide (SDS) 12% gel electrophoresis as described in methods. After transfer to a nitrocellulose membrane, CYP2E1 was revealed with rabbit polyclonal anti-hamster CYP2E1 IgG

Data represent means $\pm$ SEM; Data were analyzed by ANOVA followed by the Student Newman-Keuls post hoc test. The n is indicated in the figure.

* $p < 0.05$ HF vs. SAMe+DLPC; ** $p < 0.01$ HF vs. Control in both measurements.

Figure 2. Effect of SAME+DLPC on fat accumulation in animals treated for 3 weeks

(A) Representative histological sections of animals treated with a HF diet showed the majority of hepatocytes loaded with fat droplets (H&E $\times$ 200); (B) A section of an animal treated with the combination of SAME+DLPC showed about 40% of the hepatocytes without fat (H&E $\times$ 200). (C) Measurement of hepatic triacylglycerols showed a significant reduction of fat after 3 weeks of SAME+DLPC treatment. Liver triacylglycerol was measured by a kit obtained by DAKO according to the company specification.

Data represent means $\pm$ SEM; Data were analyzed by Student's *t* test; (n=12) in each group

Figure 3.

After three weeks of treatment, SAMe+DLPC significantly decreased hepatic CYP2E1 mRNA. Liver RNA was isolated by phenol: chloroform extraction Assessed by Northern blot using β-actin for equal loading. Three representative samples are shown for each group. Data represent means±SEM.

Data were analyzed by Student's *t* test; (n=12) in each group

Figure 4. Effect of SAME+DLPC on hepatic 4-HNE. This marker of oxidative stress was significantly reduced by SAME+DLPC

O-pentafluorobenzyl oxime trimethylsilyl ether derivatives of hepatic 4-HNE were measured by GC/MS 4-HNE was monitored at amu 152 while deuterated [$^2\text{H}_3$]-4-HNE, used as an internal standard, at amu 155. The syn-peak of 4-HNE will be eluted 0.01 to 0.02 min before the peak of the internal standard. Results were expressed in % of control Data represent means $\pm$ SEM

Data were analyzed by Student's *t* test; (n=12) in each group.

Figure 5. Effect of SAME+DLPC on hepatic TNF- α

(A) This inflammatory cytokine was significantly reduced.

50 μ l of liver lysate was used to determine the concentration of TNF- α by ELISA using the Quantikine Rat ELISA kit.

(B) There was also a significant decrease of TNF- α mRNA.

TNF- α mRNA was measured by Northern Blot as described in method

Data represent means $\pm$ SEM

Data were analyzed by Student's *t* test; (n=12) in each group.

Figure 6.

(A) The effect of SAdMe+DLPC on hepatic $\alpha 1(I)$ procollagen messenger RNA (mRNA) was measured by Northern blot. Three representative blots are shown for each group. The intensity of the bands was quantified with the Evaluating Image Analysis Systems MCID Data were expressed as a ratio of the densitometry units to β -actin.

(B) There was also a significant decrease in hepatic collagen.

Data represent means $\pm$ SEM

Data were analyzed by Student's t test; (n=12) in each group.

Table 1**Composition of the Liquid Diets**

Components	Control Diet *	High Fat Diet *
Casein	41.4	41.4
L-cystine	0.5	0.5
DL-methionine	0.3	0.3
Corn oil	8.5	48.5
Olive oil	28.4	28.4
Safflower oil	2.7	2.7
Dextrin maltose	115.2	25.6
Choline bitartrate	0.53	0.53
Fiber	10.0	10.0
Xanthan gum	3.0	3.0
Vitamins and minerals **		

*
(g/liter; 1000kcal)

**
Vitamins (/1000 kcal); thiamin hydrochloride, 1.5 mg; riboflavin, 1.5 mg; pyridoxine hydrochloride, 1.75 mg; nicotinic acid, 7.5 mg; calcium pantothenate, 4.0 mg; folic acid, 0.5 mg; biotin, 50 µg; vitamin B₁₂, 25 µg; *p*-aminobenzoic acid, 12.5 mg; inositol, 25 mg; vitamin A, 6000 IU; vitamin D, 400 IU; vitamin E, 30 IU; vitamin K, 125 µg. **Minerals** (mg/1000 kcal): calcium, 1300; phosphorus, 1000; sodium, 255; potassium, 900; magnesium, 125; manganese, 13.5; iron, 8.8; copper, 1.5; zinc, 7.5; iodine, 0.05; selenium 0.025; chromium, 0.5; chloride, 390; sulfate, 250; fluoride, 0.25 (12).

The basic diet was purchased from Dyets Inc. (Bethlehem, PA).

SAME (400mg/1000cal) and DLPC (1.5.gm/1000cal) were added and blended with all other component.

Table 2
Effects of a HF Diet with or without SAMe, DLPC and their Combination on Nutritional and Liver Parameters of Rats Treated for 6 Weeks

	Control (n=5)	HF (n=6)	HF+SAMe (n=4)	HF+DLPC (n=4)	HF+SAMe+DLPC (n=6)
Total calories	4086±150	3600±96	3958±137	4002±188	3668±102
Initial weight (g)	138.8±1.8	137.8±2.1	138.0±3.0	138.5±3.4	137.8±2.1
Final weight (g)	458.2±21.6	443.8±13.9	441.5±19.7	429.0±32.9	454.7±12.7
Weight gain/day (g)	7.8±0.5	6.9±0.3	7.1±0.5	6.8±0.8	7.1±0.3
Liver weight (g)	16.2±1.2	15.0±0.6	15.8±1.2	15.7±1.7	15.0±0.5
Liver weight (g) per 100 g BW	3.5±0.1	3.4±0.1	3.6±0.1	3.6±0.2	3.3±0.1

Data represent Means±SEM. Dietary intake, weight gain and liver weight were similar in all groups.

Table 3

Effects of a HF Diet with or without SAmE+DLPC on Nutritional and Liver Parameters of Rats Treated for 3 Weeks

	HF (n=12)	HF+SAmE+DLPC (n=12)
Total calories	1730±146	1622±125
Initial weight (g)	149.9±5.1	151.2±6.8
Final weight (g)	321.7±20.1	316.2±18.9
Weight gain/day (g)	8.2±0.9	7.9±1.1
Liver weight (g)	13.2±1.7	12.3±1.3
Liver weight (g) per100 g BW	4.1±0.4	3.9±0.2

Data represent Means±SEM in 2 groups of animals (n=12 each). Dietary intake, weight gain and liver weight were similar in both groups.

Table 4

Effect of SAmE+DLPC on Plasma Insulin, Leptin and Adiponectin of Rats Treated for 3 Weeks

	HF (n=12)	HF+SAmE+DLPC (n=12)
Insulin (ng/ml)	3.20±0.39	2.60 ±0.39
Leptin (ng/ml)	5.12±0.48	4.69±0.43
Adiponectin (µg/ml)	5.35±0.35	4.84±0.41

Data represent Means±SEM in 2 groups of animals (n=12 animals/group). Three weeks of SAmE+DLPC did not significantly affect plasma concentrations of these hormones.