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INVESTIGATION OF THE MECHANISMS OF OZONE-MEDIATED VIRAL INACTIVATION

by

Seiga Ohmine

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirement for the degree of

Master of Science

Department of Microbiology and Molecular Biology

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BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by
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This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Seiga Ohmine in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

INVESTIGATION OF THE MECHANISMS OF OZONE-MEDIATED VIRAL INACTIVATION

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Master of Science

Previous studies have established that ozone-oxygen mixtures can be used to inactivate a variety of microorganisms including bacteria, fungi and viruses. Ozone is a potent reactive oxygen species (ROS) that rapidly decays into a variety of additional short half-life ROS which have been shown to cause oxidative damage to biological molecules. I hypothesize that controlled ozone exposure and the subsequent generation of additional ROS would reduce viral infectivity by lipid and/or protein peroxidation. A proprietary ozone-oxygen delivery system was used to inactivate a series of enveloped [herpes simplex virus type-1 strain McIntyre (HSV-1), vaccinia strain Elstree (VAC), vesicular stomatitis virus strain Indiana (VSV), and influenza A strain (H1N1) A/WS/33] and non-enveloped [human adenovirus type2 (Ad2)] viruses. Plaque reduction and

suspension-infection viral antigen assays were used to determine inactivation kinetics. After ozonation, HSV-1 and VSV lost up to 6 log_{10} infectious particles in 15 min, while VAC and influenza A lost up to 5 log_{10} in 40 min and 30 min, respectively. In comparison, the non-enveloped Ad2 lost up to 5 log_{10} in 60 min. Increasing amounts of serum supplementation in the ozone treated virus suspensions slowed the rate of inactivation in both enveloped and non-enveloped viruses, suggesting the protective effect of serum against ozone. Lipid peroxidation was determined through a chromogenic assay for malondialdehyde (MDA), a byproduct of peroxidation events. MDA concentrations were inversely correlated with virus infectivity, as MDA concentrations elevated with virus exposure time to ozone. Transmission electron microscopy images of Ad2, HSV-1, VAC and VSV confirmed the drastic morphological changes that resulted from ozone treatment. The ROS-mediated attack compromised the integrity of the lipid envelopes and protein shells of the viruses. These data suggest that a wide range of viruses can be inactivated through use of an innovative ozone delivery system, thus validating my hypothesis.

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INTRODUCTION

Best known for its protective role in the atmosphere's ecological balance, the unique biological properties of ozone are currently under investigation for its potential applications in various medical disciplines. Since the latter part of the 19th century, ozone has been investigated for its reactivity with a spectrum of compounds containing unsaturated carbon-carbon double bonds (32). Through the oxidation of double bonds, ozone possesses the unique ability to destroy toxic or noxious industrial impurities (phenols, cyanides, tetraethyl lead, among others) and to inactivate biological (viral and bacterial) contaminants (5).

Ozone applications. Oxygen, one of the most fundamental elements found in nature, exists in several forms: (i) as a highly reactive and unstable single oxygen form, O^-; (ii) as atmospheric oxygen, O_2, its most stable form; (iii) highly reactive ozone, O_3; and (iv) occasionally in the form of O_4, a very rare gas which readily breaks down into atmospheric oxygen (O_2) (5). The oxidative strength of ozone is only surpassed by fluorine. Exposing ozone to organic molecules containing points of unsaturation yields many transitional compounds and further decomposed to a variety of substances (aldehydes, ketones, acids or alcohols).

The virucidal and bactericidal properties of ozone have been known since the First World War, where ozone was commonly applied topically for the disinfection of wounds and mustard gas burns (33). Following elucidation of the biochemical mechanisms involved in ozone-mediated detoxification, potential applications for ozone have greatly expanded. In July 2003, the Metropolitan Water District of Southern

California, authorized the use of ozone as the primary disinfectant at five major metropolitan treatment plants, which serve a population of over 18 million (31). Further interest in ozone has been driven by the discovery of potentially harmful by-products of chlorine disinfection.

Modern technological advances, such as ozone-mediated disinfection protocols in silicon wafer cleanroom facilities, have lead to the rapid development of ozone-inert materials used for gas delivery. From industrial technologies, medical-grade ozone generators have been developed for the delivery of ultra-pure ozone-oxygen mixtures. It has been demonstrated that the administration of autologous blood following the *ex vivo* exposure to chemical stressors such as ozone, can modulate immune responses, which is known to be critical component of atherosclerotic pathogenesis (3).

Lipid peroxidation. Lipid peroxidation (9) is a mechanism of tissue damage initiated by various ROS, which generates free radicals that are responsible for many pathological sequelae (11, 6). Lipids contain many points of unsaturation along their hydrocarbon chains, and oxidative deterioration of these phospholipids lead to severe structural and functional damage to the lipid bilayer of the plasma membrane. Other common cellular lipid constituents include cholesterol, triglycerides and free fatty acids, all of which can be readily oxidated through lipid peroxidation. In whole blood, the total lipid concentration is approximately 4.5 to 10 mg/ml, providing many potential targets for ozone-mediated lipid peroxidation (16, 40).

Singlet oxygen, hydroperoxides, hydrogen peroxide, ozonides, carbonyls, alkanes and alkenes are just some of the products of lipid peroxidation. Peroxides from polyunsaturated fatty acids generate malondialdehyde (MDA) and 4-hydroxyalkenals

upon decomposition. A widely accepted standard for measuring lipid peroxidation has been an assay for MDA, a stable peroxidation byproduct (11).

Innate biologic defense mechanisms of the body exemplify the damaging effects of lipid peroxidation. Vitamin E, which includes the tocopherols and the tocotrienols (15), has been hypothesized to play a central role in scavenging potentially damaging ROS (29, 37). Reactive lipid peroxides are scavenged when vitamin E is present in the membrane or lipid domains. Through this process, vitamin E acts as the main antioxidant that protects the lipid membrane from peroxidation (29). Enzymes such as superoxide dismutase, catalase and glutathione peroxidase are just some of the other nascent mechanisms for protection against lipid peroxidaiton (30).

Protein peroxidation. Protein peroxidation is defined as the covalent modification of a protein through either direct interaction by reactive oxygen species or indirect interaction by reaction with secondary by-products of oxidative stress (38). Various ozone-mediated ROS can interact with proteins and cause oxidative changes to inhibit normal cellular mechanisms, such as the losses of aggregation and proteolysis control, changes in enzyme-substrate binding activities, and alterations in immunogenicity (38). In particular, protein peroxidation may play a key role in the inactivation of non-enveloped viruses, such as adenovirus, poliovirus and other enteroviruses. Some prominent amino acids that are targets of peroxidation include the following: cysteine (disulfides and mixed disulfides), methionine (sulfoxides), tyrosine (di- and nitro-tyrosine), tryptophan (hydroxytryptophan), histidine (2-oxohistidine and asparagines) and lysine (chloramines, MDA-lysine and carboxymethyllysine).

DNA damage through peroxidation. The lipid peroxidation byproducts, such as MDA and 4-hydroxy2-nonenal, are particularly potent in forming DNA adducts during oxidative stress. DNA adducts play crucial roles in tumorigenesis, as many types of human cancers are implicated in areas of persistent oxidative stress (26). Through DNA adduct formation, tumor suppressor gene expression may be inhibited and oncogenes may be activated. These increases in genomic instabilities and mutation frequencies can eventually lead to malignancy. The prevention of DNA adduct formation may play a crucial role in the development of cancer prevention strategies (17).

Ozone and viruses. Currently, two main applications for ozone-mediated viral inactivation are appreciated. First, there was an abundance of research performed in the latter half of the 20th century which involves the ozone-mediated inactivation of many water-contaminating viruses. Second, there were many attempts at ozone-based therapy modalities for the treatment of viremia. However, a precision delivery system has been lacking, and the toxicity produced by the ROS bombardement has limited the progression of ozone applications in medicine.

Norwalk viruses and other human caliciviruses, which lack a lipid envelope, are major agents of epidemic gastroenteritis, and water is a critical component of its widespread transmission. Studies suggested that Norwalk viruses were very resistant to free-chlorine disinfection (24), and ozone was a safer alternative since it had rapidly reduced Norwalk virus infectivity as well (39). Possible targets for peroxidation include the protein capsid and viral antireceptors, which are both critical for proper viral adsoption into the host cell.

Alternatively, singlet oxygen species that have been generated through ozone-mediated oxidation have been known to inactivate virus while leaving the capsid intact, and in the case of poliovirus, research has indicated that genomic integrity may have been compromised through ROS attack (13). Although ozone was shown to disrupt the polypeptide chains on the viral protein coat of poliovirus-1, this did not significantly affect the adsorption of the virus particle into the host cell (35). Through velocity sedimentation analysis, it was shown that the viral nucleic acid was extensively damaged, and inactivation was primary through this mechanism (35). In related studies, poliovirus-3 and MS2 coliphage have been inactivated through ozone-mediated chemical disinfection (12), and ozonated water was mixed with a poliovirus-1 suspension using a fast-flow mixer, which inactivated 95 to 99% of the virus (22).

Enveloped viruses, such as human immunodeficiency virus type-1 (HIV-1) have demonstrated sensitivity to ozone treatment. In a study performed in 1991, an 11 $\log_{10}$ reduction in HIV-1 was achieved within 2 hours (43). In another study, Carpendale and Freeberg demonstrate that ozone can inactivate HIV at non-cytotoxic concentrations (7). Venezuelan Equine Encephalomyelitis virus has also been successfully inactivated using a liquid-phase ozone application (1). The infectivity of this arboviral pathogen was reduced 7 $\log_{10}$ in approximately 45 min (1).

Viral components necessary for proper infection, such as the membrane-associated antireceptors and lipid envelope, may have been primary targets of ROS attack. The sensitivity of lipid-containing viruses was exposed, indicating that the virus may lose its infectivity through lipid envelope peroxidation. Through interactions with complex carbohydrates, glycoproteins and sphingolipids, ozone generates reactive oxygen species

(ROS) such as singlet oxygen and lipid peroxides (35). This is of particular interest due to the variety of lipid components in whole blood (16), and in the presence of ROS, these proteins and lipids may play a protective role in virus inactivation. The primary mechanism of virus inactivation is believed to be through these ozone-generated ROS.

In 1996 a research team suggested the application of a nebulization technique to inactivate viruses with ozone (23). In their studies, this nebulization technique was successful in the inactivation of viruses with ozone in large volumes of body fluids (eg plasma, partial blood and whole blood) in a relatively short period of time.

Adenovirus type-2. Adenoviruses, a member of the *Adenoviridae* family, were first identified in the adenoids while searching for the etiologic agents of acute human respiratory infections (36). Currently, over 100 members of the adenovirus family that infect a wide range of mammalian and avian hosts have been identified. All adenoviruses contain a linear, double-stranded DNA genome encapsidated in an icosahedral protein shell, with particles measuring 70 to 100 nm in diameter (18). Adenoviruses consist of a tough protein shell surrounding a DNA core (18). A characteristic feature of adenoviruses is the presence of 12 fibrous proteins on each virus particle which project from the center of penton capsomeres located at the vertices of the icosahedral capsid (36). Adenovirus type-2 (Ad2) was the first adenovirus to be completely sequenced (34), and it totals to 36 kbp. Ad2 also has an inverted terminal repeat sequence that varies in length (40 to 160 bp), and this sequence allows the formation of a panhandle structure, which is a crucial intermediate structure in the replication cycle of the virus (2, 42).

Herpes simplex virus type-1. Herpes simplex virus type-1 (HSV-1) is a member of the *Herpesviridae* family. HSV-1 consists of a glycoprotein-containing lipid envelope that surrounds tegument proteins and an icosehedral nucleocapsid. The nucleocapsid that contains the viral genome is approximately 110 nm, and the genome is a linear double-stranded DNA that varies in length from 120 to 250 kbp. The viral DNA is contained in the nucleocapsid in the form of a torus, which is similar to a large convex molding, and semicircular in cross section (14). 162 capsomeres comprise the herpesvirus nucleocapsid (44). Upon negative staining, an amorphous fibrous network may be observed between the envelope and the capsid. Termed the tegument, it is an ordered structure that displayed structural polarity upon visualization through immunoelectron microscopy (41). The viral envelope contains many short glycoprotein extensions; HSV-1 envelopes contain at least 11 types of glycoproteins which can be present in high numbers, often exceeding 1000 per virion (44).

Influenza. A member of the family *Orthomyxoviridae*, influenza is an enveloped virus which contains a segmented, negative-stranded RNA genome. Influenza A and B viruses each contain eight segments of negative-stranded RNA, and influenza C viruses lack a neuraminidase gene, therefore only containing seven segments (25). The lipid envelope of influenza is comprised of host cell-derived plasma membrane (8), and viral transmembrane glycoprotein projections. An interesting feature of the influenza A virion is a layer of about 500 glycoprotein projections from the lipid envelope, which are about 10 to 14 nm in length. Viral-encoded hemagglutinin (HA) and neuraminidase (NA) comprise these projections, and are usually found in the respective ratios of 4:1 to 5:1 (8).

Vaccinia virus. Unlike most other DNA viruses, vaccinia virus, a member of the *Poxviridae* family, replicates in the cytoplasm of the host cell, and the proteins they encode permit cytosolic viral replication and viral gene expression. The vaccinia genome is composed of a single, linear double-stranded DNA approximately 130 to 300 kbp in length. Inverted terminal repeat sequences flank either end of the vaccinia genome, creating a hairpin loop. The general structure of this large virus can be visualized under a light microscope, and further details of the virus's complexity can be visualized through electron microscopy. Measuring approximately 350 nm in length and 270 nm in width, these viruses appear as smooth, rounded rectangles. Negatively stained images of the poxvirus core have a brick-like appearance; the wall of the core appears to be composed of an outer layer of cylindrical subunits 10 nm in length and 5 nm in diameter (10).

Vesicular stomatitis virus. Vesicular stomatitis virus (VSV) is the best-studied member of the family *Rhabdoviridae*. Rhabdoviruses are simple non-segmented negative-stranded RNA viruses. Due to the negative-stranded nature of the genome, the virus genome encodes an RNA-dependent RNA polymerase, which was the first such polymerase to be identified (4). The genome is approximately 12 kb in length, and is tightly associated with a ribonucleoprotein complex that is coiled into a helical nucleocapsid. This nucleocapsid is tightly associated with the viral envelope, creating a bullet-shaped appearance. VSV particles are typically 180 nm long and 70 nm wide, however certain plant rhabdoviruses have more filamentous shape that can be up to 400 nm long (20). The viral envelope is derived from its host cell, and viral transmembrane glycoprotein structures can be easily visualized through electron microscopy images.

Current literature suggests that many enteroviruses that use water as a means of transmission can be readily inactivated through ozone treatments. However, clinical applications of ozone treatment in medicine have been limited due to the lack of data supporting the successful delivery of non-cytotoxic ozone dosages. If this ozone-oxygen gas mixture could be delivered in a precise, measured dosage, the beneficial virucidal properties of ozone may be seen at non-cytotoxic concentrations. The hypothesis tested in this study was to determine if a precision delivery of an ozone-oxygen gas mixture could inactivate a variety of viruses (enveloped, non-enveloped, DNA/RNA, simple, complex etc.) using a minimal amount of ozone gas, thus maintaining the biological integrity of the treated fluid. This precision delivery protocol may allow for widespread applications for ozone treatments, including improved water sanitation, serum product disinfection and serve as a primer for clinical studies involving ozone-mediated viremia treatments.

MATERIALS AND METHODS

Cell culture. Vero (African green monkey kidney) cells obtained from the American Type Culture Collection (ATCC, Manassas, MD) were propagated in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen Corporation, Carlesbad, CA) supplemented with 5% cosmic calf serum (CCS) (Hyclone, Logan, UT), 2.0 mM GlutaMAX (Invitrogen Corporation), 40 µg/ml gentamicin and 200 ng/ml fungizone/amphotericin B. Cells were incubated at 37 ^{o}C with 5% CO_2.

Virus stock preparation. Herpes simplex virus type-1 (HSV-1, strain: McIntyre), vesicular stomatitis virus (VSV, strain: Indiana) and Vaccinia virus (VAC, strain: Elstree) infections were carried out in Vero cells. HSV-1 was obtained from Dr. F.B. Johnson, and VSV and VAC were obtained from the ATCC. Confluent flasks of Vero cells were washed once with Dulbecco's phosphate buffered saline (DPBS, calcium and magnesium free) for 5 min, then infected at a multiplicity of infection (MOI) of 5-10 plaque-forming units (PFU) per cell. The virus inoculum was allowed to adsorb to the cells for 1 h at 36 ^{o}C with 5% CO_2. 10 ml of medium 199 supplemented with 2% heat-inactivated newborn calf serum, 2.0 mM GlutaMAX, 40 µg/ml gentamicin and 200 ng/ml fungizone/amphotericin B was added to each flask, and incubated at 36 ^{o}C with 5% CO_2. Virus-infected cells were harvested at >90% cytopathic effect (CPE) through two freeze-thaw cycles using a -80 ^{o}C freezer, and centrifuged at 1000 x g for 10 min to clarify cellular debris.

Standard plaque assay. Standard plaque assays were carried out in Vero cells for HSV-1, VSV and VAC. Vero cells were seeded at a concentration of 2.75 x 10^5

cells/ml/well in 24-well plates, and incubated for 24 h at 37 ^oC. Serial dilutions of viruses were prepared in DMEM supplemented with 2% fetal bovine serum (FBS) (Hyclone, Logan, UT), 2.0 mM GlutaMAX, 40 μg/ml gentamicin and 200 ng/ml fungizone/amphotericin B. After removal of the culture media, the virus inoculum was allowed to adsorb to the cells for 1 h at 36 ^oC with 5% CO_2. Following incubation, 1.0 ml of Minimal Essential Medium (MEM) supplemented with 2% FBS, 1% (w/v) methylcellulose, 2.0 mM GlutaMAX, 40 μg/ml gentamicin and 200 ng/ml fungizone/amphotericin B was added to each well. Plates were incubated in 36 ^oC with 5% CO_2 until visual confirmation of plaques under the microscope. Cells were fixed with 10% formaldehyde (v/v) solution in ddH_2O for 45 min, and stained with 1% (w/v) crystal violet solution in ddH_2O for 30 min. Plaques were counted under the microscope, and virus titers were reported as plaque-forming units (PFU) per ml.

Suspension infection. HeLa cells obtained from the ATCC were propagated in DMEM supplemented with 10% FBS, 2.0 mM GlutaMAX, 10 mM HEPES buffer, 40 μg/ml gentamicin and 200 ng/ml fungizone/amphotericin B. HeLa cells for the suspension infection cultures were prepared by standard trypsinization. The loosened cell sheets were suspended in culture medium (10 ml/75 cm^2 flask), and placed on ice until infection. 2.0 ml of culture medium, 0.2 ml of HeLa suspension and 0.1 ml of virus dilution were incubated for 18-24 hours at 35-36 ^oC in a flat-sided polystyrene Nunclon™ tube (Nalge-Nunc International, Rochester, NY). Samples were fixed for 15 min in FAA fixative (5% formalin, 5% glacial acetic acid and 80% ethanol contained in distilled water) followed by 30 min incubations in each of three reagents supplied by Dr. F.B. Johnson (27). The first reagent contained a primary Ad2 antibody with blocking

reagent, the second reagent contained the enzyme conjugate and the third reagent contained the substrate-chromogen reagent consisting of 0.56 mM 4-chloro-1-naphthol with 0.003% hydrogen peroxide in ddH_2O (27, 21). Tubes were washed with distilled water between reagent incubations. Stained cells were counted under the microscope, and corresponding virus titers were calculated as focus-forming units (FFU) per ml.

Ozonation of viruses. High titer virus stocks (>1.0 x 10^8 PFU/FFU per ml) were diluted into DPBS with varying concentrations of cosmic calf serum (0%, 10%, 50%, 80% or 100% serum) into a total volume of 140 ml. The fluid was injected into the proprietary Gas-Fluid exchange device (Lipidviro Tech, Inc., Salt Lake City, UT), which produced a turbulent, thin-layer liquid film for maximum ozone exposure. The proprietary Drug Production and Delivery system (Lipidviro Tech, Inc.), produces ozone gas from medical grade pure oxygen and delivered ozone to the Gas-Fluid exchange device containing the virus suspension. 1200 parts per million per volume (ppmv) of ozone was used, and the oxygen gas injection rate was 2000 ml/min while the fluid pump rate was 252 ml/min. At multiple time points, 1.5 ml samples were removed from the device so viral infectivity could be measured. Viral infectivity of HSV-1, VSV and VAC were measured using the standard plaque assay, while Ad2 infectivity was determined using the suspension-infection antigen assay. Influenza virus infectivity was assayed in the laboratory of Dr. F.B. Johnson using Madin-Darby Canine Kidney (MDCK) cells and foci of infected cells were identified by immunofluorescence.

Lipid peroxidation assay. A malondialdehyde (MDA) standard and lipid peroxidation assay kit (Oxford Biomedical Research, Oxford, MI) was used to determine the lipid peroxidation levels in ozone-conditioned virus suspensions. The 10 mM MDA

stock solution was diluted 1/500 into ddH_2O to yield a 20 µl stock solution. A standard curve was generated by using the dilution scheme in Table 1. One volume of n-methyl-2-phenylindole was diluted into three volumes of methanol, and 650 µl of the dilution was added to 200 µl of the test sample in a sterile microcentrifuge tube. After gentle vortexing, 150 µl of 12 N HCl was added and the test samples were incubated at 47 ^{o}C for 1 h. Samples were centrifuged at 15,000 x g for 10 min and the supernatant was collected in a cuvette. Absorbance was measured at 586 nm using a spectrophotometer.

Transmission electron microscopy. Carbon-coated copper grids (Electron Microscopy Sciences, Hatfield, PA) were coated with 0.25% formvar in ethylene chloride, and placed on top of 1% agarose. 40 µl of prepared virus samples were placed on the copper grid and samples were allowed to diffuse for over 6 h at 4 ^{o}C. Samples were negatively stained with 1.5~2.0% phosphotungstic acid (PTA) (Electron Microscopy Sciences) at pH 5.2 for 5 min. Samples were viewed in an FEI Tecnai-12 transmission electron microscope set at 120 kV. HSV-1 and vaccinia virus samples were viewed at the diluted concentrations used for ozone treatment. 14.0 ml of Ad2 and VSV were concentrated by centrifugation at 80,000 x g for 4 h at 10 ^{o}C in the Beckman L8-60M ultracentrifuge with the SW28 rotor. Supernatant was decanted and the viruses were re-suspended in 500 µl of DPBS.

RESULTS

The following results for ozone-mediated viral inactivation are presented alphabetically by virus species.

Ozone mediated inactivation of adenovirus type-2. In order to confirm that viral inactivation was ozone-dependent, Ad2 was suspended in 100% DPBS and treated with O_2 gas under conditions previously described in Materials and Methods. 1.5 ml samples were removed at 0, 30 and 60 min for viral infectivity assay, each time point on the graph (Fig. 1) is representative of three replicates of one ozone treatment run. Ad2 titers were reduced from 2.03×10^7 FFU/ml to 1.89×10^7 FFU/ml in 60 min (Fig. 1).

Ad2 was treated with ozone as previously described. 1.5 ml samples were removed at time 0, 20, 40 and 60 min to determine viral infectivity. Viral infectivity data presented are representative of two replicates from two independent ozone conditioning runs. Ad2 was suspended in 100% DPBS and conditioned with ozone, 2.41×10^2 FFU/ml of infectious virus particles remained after 60 min of ozone conditioning from a starting titer of 2.36×10^7 FFU/ml (Fig. 1). When Ad2 was suspended in 50% CCS in DPBS, 2.45×10^7 FFU/ml of infectious virus particles were reduced to 1.21×10^4 FFU/ml following 60 min of ozone conditioning (Fig. 1). Similarly, a reduction from 2.41×10^7 FFU/ml to 1.37×10^4 FFU/ml of infectious virus particles were seen following 60 min of ozone treatment when Ad2 was suspended in 100% CCS (Fig. 1).

Lipid peroxidation assay of ozone-treated adenovirus type-2. Ozone-treated samples from Ad2 suspended in 100% DPBS were used to measure MDA content. An

MDA standard curve (Fig. 2) was used to derive the MDA content of 0, 20 and 60 min samples of ozone treated Ad2. 1.04 µM of MDA was detected at time 0, and ozone treatment increased MDA concentration to 15.26 µM and 21.31 µM in 20 and 60 min, respectively (Fig. 3).

Transmission electron microscopy images. Ad2 samples were negatively stained with PTA and transmission electron microscopy images were taken at 120 kV. Untreated Ad2 samples are shown in the control images (Fig. 4 and 5). The intact Ad2 protein capsid is clearly seen and the icosehedral Ad2 capsid's two-fold axis of symmetry is seen in detail (Fig. 5B). Ad2 samples shown in Figure 6 were treated with 1200 ppmv ozone, and the disruption of the Ad2 protein capsid can be seen (Fig. 6 A and B). Image in Figure 6B was further enlarged with Adobe Photoshop.

Ozone mediated inactivation of herpes simplex virus type-1. To confirm that viral inactivation was mediated by ozone exposure, HSV-1 was suspended in 100% DPBS and treated with O_2 gas under conditions previously described. 1.5 ml samples were removed at 0, 20, 40 and 60 min for viral infectivity assays, each time point on the graph (Fig. 7) is representative of four replicates of one ozone treatment run. HSV-1 titers were reduced from 2.40 x 10^6 PFU/ml to 1.73 x 10^5 PFU/ml in 60 min (Fig. 7).

HSV-1 was treated with ozone under the conditions previously described. Viral infectivity data presented are representative of four replicates from two independent ozone treatment runs. When HSV-1 was suspended in 100% DPBS, 1.5 ml samples were removed at time 0, 5, 10 and 15 min to determine viral infectivity. 3.92 x 10^1 PFU/ml of infectious virus particles remained after 15 min of ozone conditioning from a starting titer of 1.17 x 10^7 PFU/ml (Fig. 7). For CCS supplemented HSV-1 treatments, 1.5 ml samples

were removed at time 0, 15, 30, 45 and 60 min to determine viral infectivity. When HSV-1 was suspended in 10% CCS in DPBS, a virus titer of 2.48×10^6 PFU/ml was completely inactivated in 45 min (Fig. 7). Similarly, a virus titer of 2.73×10^6 PFU/ml was completely inactivated in 45 min when the virus was suspended in 50% CCS in DPBS (Fig. 7). When HSV-1 was suspended in 80% CCS in DPBS, complete inactivation of 2.11×10^6 PFU/ml was seen in 60 min (Fig. 7). HSV-1 titers were reduced from 1.41×10^6 PFU/ml to 7.50×10^1 PFU/ml in 60 min in 100% CCS (Fig. 7).

Lipid peroxidation assay of ozone-treated herpes simplex virus type-1. Ozone-treated samples from HSV-1 suspended in 100% DPBS were used to measure MDA content. An MDA standard curve (Fig. 8) was used to derive MDA content of ozone conditioned HSV-1. No MDA was detected at time 0, and ozone treatment increased MDA concentration to 11.99 μM, 13.40 μM and 16.36 μM in 5, 10 and 15 min, respectively (Fig. 9).

Transmission electron microscopy images of herpes simplex virus type-1. HSV-1 samples were negatively stained with PTA and transmission electron microscopy images were taken at 120 kV. Untreated HSV-1 samples are shown in the control images (Fig. 10 and 11). The intact HSV-1 icosehedral nucleocapsid is clearly seen and three-fold axis of symmetry is visualized in detail (Fig. 11 A and B). HSV-1 samples shown in Fig. 12 were suspended in 100% DPBS and treated with ozone for 15 min. The complete destruction of the viral envelope can be observed (Fig 12 A, B and C) and the altered morphology of the icosehedral nucleocapsid suggests severe ROS-mediated damage.

Ozone mediated inactivation of influenza A. Influenza A was suspended in DME with 0.125% bovine serum albumin, and treated with 1200 ppmv of ozone under

conditions previously described. The starting titer of the virus suspension was 3.16×10^5 focus forming units per ml (FFU/ml). 1.5 ml samples were removed at 0, 15, 30 and 60 min. Assays for viral infectivity were performed in Dr. F. B. Johnson's laboratory using MDCK cells and foci of infected cells were identified by immunofluorescence. A 3.0 $\log_{10}$ loss of viral infectivity was seen in 15 min and no infectious particles were detected in the 30 min sample and the 60 min sample (Fig. 13). A total of 3.16×10^5 of infectious influenza A particles were inactivated in 30 min (Fig. 13). Results are from one ozone treatment run.

Ozone mediated inactivation of vaccinia virus. To confirm that viral inactivation was mediated by ozone exposure, VAC was suspended in 100% DPBS and treated with O_2 gas under conditions previously described. 1.5 ml samples were removed at 0, 20, 40 and 60 min for viral infectivity assay, each time point on the graph is representative of four replicates of one ozone treatment run (Fig. 14). VAC titers were reduced from 4.62×10^5 PFU/ml to 1.30×10^5 PFU/ml in 60 min (Fig. 14).

VAC was treated with 1200 ppmv of ozone. Viral infectivity data presented are representative of four replicates from two independent ozone condition runs. When VAC was treated in 100% DPBS, 1.5 ml samples were removed at time 0, 20, 30 and 40 min to determine viral infectivity. VAC titer was completely inactivated from 5.13×10^5 PFU/ml in 30 min (Fig. 14).

For 10% and 50% CCS supplemented VAC treatments, 1.5 ml samples were removed at time 0, 15, 30, 45 and 60 min to determine viral infectivity. When VAC was treated in 10% CCS in DPBS, a virus titer of 3.06×10^5 PFU/ml was completely inactivated in 60 min (Fig. 14). In 50% CCS in DPBS, a virus titer of 4.18×10^5 PFU/ml

was completely inactivated in 60 min (Fig. 14). Finally, 1.5 ml samples were removed at time 0, 20, 40 and 60 min to determine viral infectivity for VAC ozone treatment in 100% CCS. Virus titers were reduced from 3.22 x 10^6 PFU/ml to 1.45 x 10^3 PFU/ml in 60 min. When VAC was suspended in 80% CCS in DPBS and 100% CCS, complete inactivation of 1.16 x 10^6 PFU/ml was seen in 60 min (Fig. 14).

Lipid peroxidation assay of ozone-treated vaccinia virus. VAC samples suspended in 100% DPBS were treated under conditions of 1200 ppmv of ozone and sampled to measure MDA content. An MDA standard curve (Fig. 15) was used to derive MDA content of ozone conditioned VAC. 0.115 µM of MDA was present at time 0, and following ozone treatment, MDA concentrations increased to 9.0 µM and 10.71 µM in 20 and 60 min, respectively (Fig. 16).

Transmission electron microscopy images of vaccinia virus. Negatively stained (PTA) transmission electron microscopy images of VAC were taken at 120 kV. Untreated VAC particles are shown in Fig. 17. VAC electron micrographs shown in Fig. 18 were treated with 1200 ppmv ozone for 40 min. Images suggest severe disruption of normal “rounded rectangle” VAC morphology.

Ozone mediated inactivation of vesicular stomatitis virus. To confirm that viral inactivation was mediated by ozone exposure, VSV was suspended in 100% DPBS and treated with O_2 gas under conditions previously described. 1.5 ml samples were removed at 0, 20, 40 and 60 min for viral infectivity assay, each time point on the graph (Fig. 19) is representative of four replicates of one ozone treatment run. VSV titers were reduced from 1.36 x 10^6 PFU/ml to 2.33 x 10^5 PFU/ml in 60 min (Fig. 19).

VSV was treated with 1200 ppmv of ozone. Viral infectivity data presented for 100% DPBS, 10% CCS and 50% CCS supplemented DPBS virus suspensions are representative of four replicates of three independent ozone condition runs. When VSV was treated in 100% DPBS, 1.5 ml samples were removed at time 0, 5, 10 and 15 min to determine viral infectivity. 1.83×10^6 PFU/ml of infectious VSV particles were completely inactivated within 15 min of ozone treatment (Fig. 19). For 10% CCS supplemented VSV treatments, 1.5 ml samples were removed at time 0, 10, 20, 30 and 40 min to determine viral infectivity. A VSV titer of 1.74×10^6 PFU/ml was completely inactivated in 40 min (Fig. 19). When VSV was treated in 50% CCS supplemented DPBS, 1.5 ml samples were removed at time 0, 20, 40 and 60 min to determine viral infectivity. Complete inactivation of VSV was achieved from a titer of 2.46×10^6 PFU/ml in 60 min (Fig. 19). 80% CCS supplemented and 100% CCS VSV ozone treatments were performed in four replicates of two independent ozone runs. For VSV suspended in 80% CCS in DPBS, 1.5 ml samples were removed for assay at times 0, 20, 40 and 60 min. A titer of 2.19×10^6 PFU/ml was completely inactivated within 60 min (Fig. 19). For VSV suspended in 100% CCS, 1.5 ml samples were removed at 0, 15, 30, 45 and 60 min. 8.56×10^5 PFU/ml of VSV was reduced to 3.13×10^1 PFU/ml in 60 min (Fig. 19).

In another series of experiments, VSV was suspended in 100% CCS and treated with 1000 ppmv and 1500 ppmv of ozone. VSV titer in 1000 ppmv of ozone was reduced from 2.75×10^5 PFU/ml to 3.75×10^2 PFU/ml over 60 min (Fig. 20), while VSV treated with 1500 ppmv of ozone completely inactivated a starting titer of 4.05×10^5 PFU/ml in 45 min (Fig. 20). Data are representative of four replicates of one ozone run.

Lipid peroxidation assay of ozone-treated vesicular stomatitis virus. VSV samples suspended in 100% DPBS were treated for 15 min at 1200 ppmv, and used to measure MDA content. An MDA standard curve (Fig. 21) was used to derive MDA content of ozone treated VSV. Before ozone treatment, 0.65 µM of MDA was present, and following treatment, MDA concentrations increased to 6.13 µM and 10.145 µM in 5 and 15 min, respectively (Fig. 22).

Transmission electron microscopy images of vesicular stomatitis virus. Negatively stained (PTA) transmission electron microscopy images of VSV were taken at 120 kV. Untreated VSV are shown in Fig. 23. The normal bullet-shaped morphology of VSV is visible (Fig. 23). VSV samples shown in Fig. 24 were treated with 1200 ppmv of ozone for 15 min. These TEM images suggest disruption of normal "bullet-shaped" VSV morphology.

All statistical analysis and graphing were performed in an MS Excel spreadsheet.
A summary of all data are presented in Table 2.

DISCUSSION

Currently in the United States, ozone-mediated viral inactivation applications are limited to disinfections at water treatment municipalities (31). Thus far, FDA approval of clinical ozone treatment protocols for viremia has not been forthcoming. Although ozone treatment protocols have been successful in the inactivation of several enteroviruses (7, 23, 12 and 22), clinical experiments have not yielded equivalent success.

The major insufficiency with current ozone-mediated inactivation protocols lies in the inability to precisely measure and control ozone-oxygen gas delivery. Cytotoxicity of ozone-treated liquids (23) creates an enormous obstacle for clinical applications of ozone. Although success has been reported with the inactivation of some notorious pathogens such as Venezuelan Equine Encephalitis virus, an arthropod-borne alphavirus endemic to northern South America, Trinidad, Central America, Mexico and Florida, its applications were limited to biological safety cabinet and laboratory equipment sterilization (1).

The results of this experiment support my hypothesis that a wide variety of viruses can be inactivated when exposed to controlled amounts of ozone. Viruses suspended in 100% DPBS were rapidly inactivated at 1200 ppmv of ozone gas (Fig. 1, 7, 13, 14 and 19). When viruses were suspended in DPBS supplemented with serum, the effects of ozone were buffered by the abundant proteins in serum, and slowed the kinetics of viral inactivation (Fig. 1, 7, 13, 14 and 19). Enveloped viruses, such as HSV-1 (Fig. 7), VSV (Fig. 19) and Influenza A (Fig. 13), showed extreme sensitivity to ozone, and under the same conditions, the complex VAC virus was rapidly inactivated as well (Fig. 14). However, the non-enveloped Ad2 showed the most resistance to ozone treatments (Fig.

1). These data support the idea that the rate of viral inactivation is dependent on direct ozone contact (19).

Elevated lipid peroxidation levels, as indicated by higher MDA concentrations, suggested an abundance of primary and secondary ROS that may aid in viral inactivation. As expected, MDA concentrations increased over ozone treatment time (Fig. 3, 9, 16 and 22), and were inversely related to infectious virus titer. Potential targets for ROS attack included the envelopes of viruses such as HSV-1, VSV, Influenza A and VAC and the protein shells of Ad2 through lipid and protein peroxidation.

Transmission electron microscopy images displayed the dramatic ozone-mediated changes of virus morphology. TEM images from ozone-treated HSV-1 demonstrated the sensitivity of the lipid envelope to ROS attack (Fig. 12), and destruction of the icosehedral nucleocapsid was clearly visible (Fig. 12). VAC also demonstrated dramatic changes upon ozone treatment (Fig. 18), and visible morphological changes could be seen with ozone treated Ad2 (Fig. 6) and VSV (Fig. 24).

The applications of the unique biological properties of ozone have been limited thus far. Previous studies lack the technology to deliver controlled amounts of ozone into a system. However, our data suggest that through precision control of ozone using this proprietary ozone-oxygen delivery system, the toxic effects of ozone can be minimized while maximizing the beneficial virucidal and bactericidal properties of ozone. Real-time monitoring and controlling of ozone gas flow, ozone absorption and ozone-oxygen gas exposure provide for an ozone-oxygen delivery system with an unprecedented level of control. Using this technology, significant improvements could be made for water disinfection protocols, serum product sterilization methods and treatments for

athlerosclerosis (3) and viremia. These results provide a basis for further studies involving the ozone-mediated inactivation of viruses and offers opportunity for the inactivation of other infectious materials.

TABLE 1. MDA standard curve dilution volumes.

Target concentration of standard in reaction mixture, µM	0	0.50	1.00	2.00	3.00	4.00
Volume of 20 µM standard to add	0	25	50	100	150	200
Volume of water or buffer to add	200	175	150	100	50	0

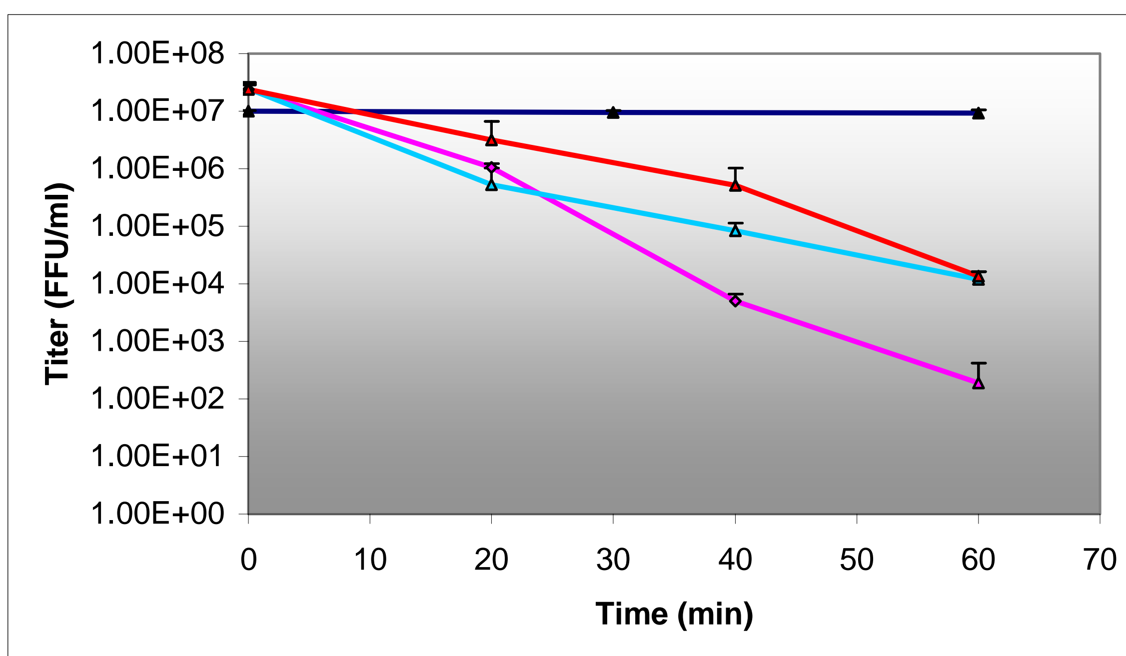


FIGURE 1. Inactivation of adenovirus type-2 when treated with 1200 ppmv ozone. Ad2 was treated under the conditions of oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. (▲) Ad2 suspended in 100% DPBS with O_2 gas only. Points represent the averages of three replicates in one ozone treatment run. For the following, data points represent the averages of two replicates in two separate ozone treatment runs. (▲) Ad2 suspended in 100% DPBS treated with 1200 ppmv ozone. (▲) Ad 2 suspended in 50% CCS in DPBS with 1200 ppmv ozone. (▲) Ad2 suspended in 100% CCS treated with 1200 ppmv ozone. All y-axis error bars represent one standard deviation from the mean.

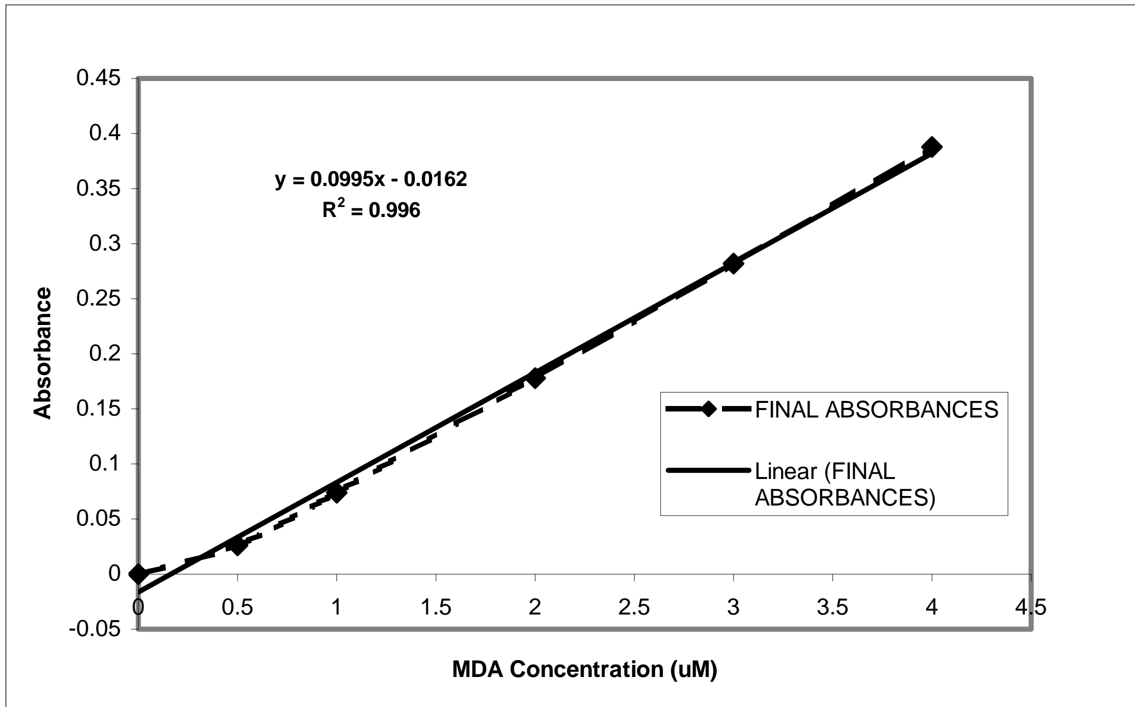


FIGURE 2. Standard curve of lipid peroxidation assay for adenovirus type-2. This standard curve was used to derive the MDA concentrations found in ozone conditioned Ad2 suspended in 100% DPBS (Fig. 3).

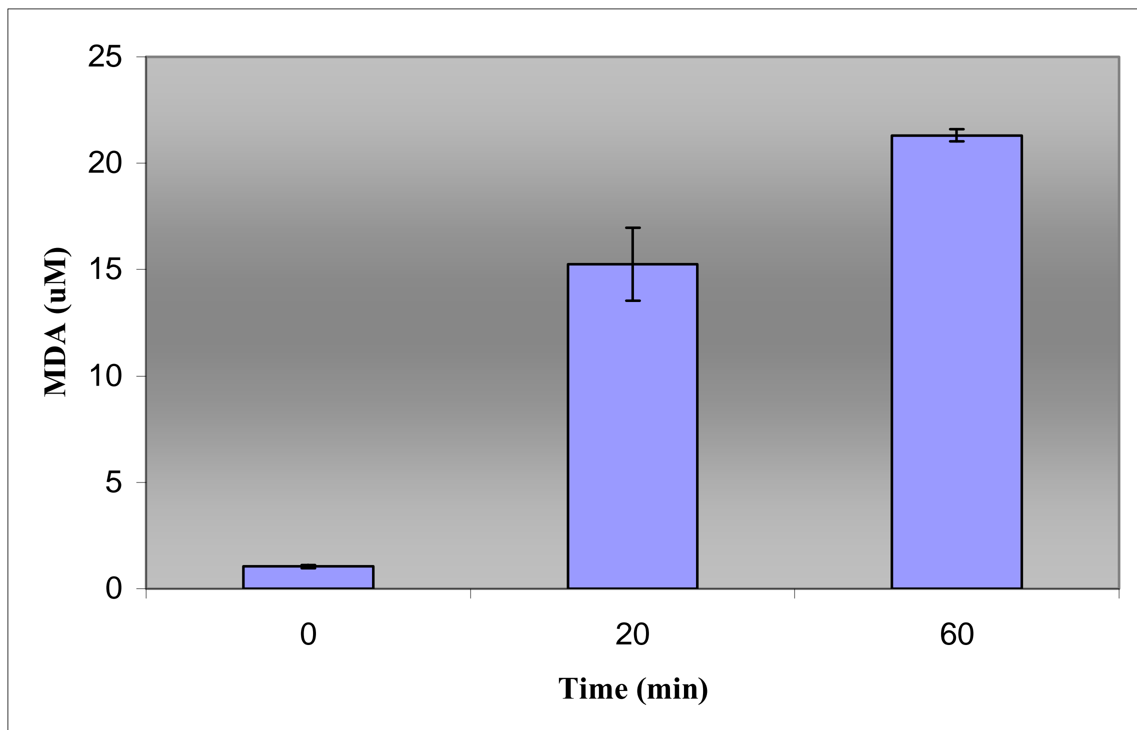


FIGURE 3. MDA concentrations of adenovirus type-2 suspended in 100% DPBS, treated with 1200 ppmv ozone. Ad2 was suspended in 100% DPBS, and treated under the conditions of 1200 ppmv of ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. 0 min, 20 min and 60 min ozone treatment samples were assayed for MDA content and reported in µM. Data are representative of two replicates runs from the same standard curve (Fig. 2). Error bars represent one standard deviation from the mean.

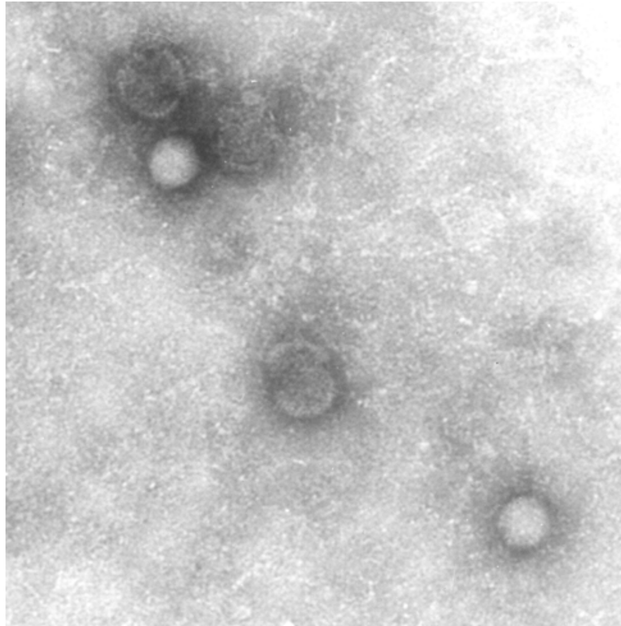


FIGURE 4. Transmission electron micrograph analysis of untreated adenovirus type-2 suspended in 100% DPBS (1). Untreated Ad2 was negatively stained with PTA and micrographs were taken at 52,000 X and 120 kV.

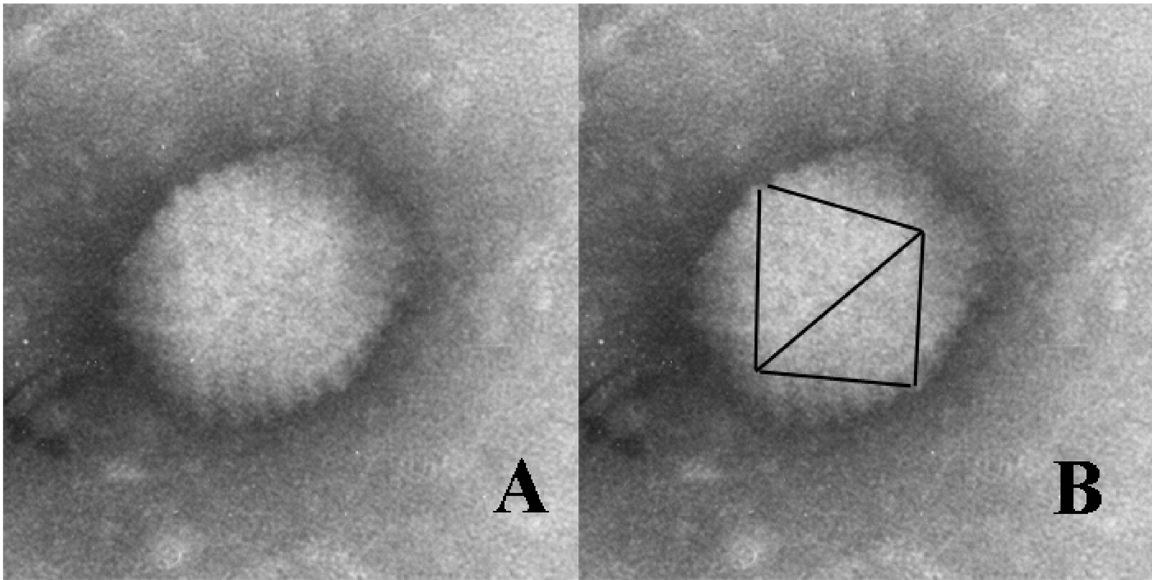


FIGURE 5. Transmission electron micrograph analysis of untreated adenovirus type-2 suspended in 100% DPBS (2). Untreated Ad2 was negatively stained with PTA and micrographs were taken at 150,000X and 120 kV. A two-fold axis of symmetry on the Ad2 capsid can be seen (A) and axis is outlined in (B).

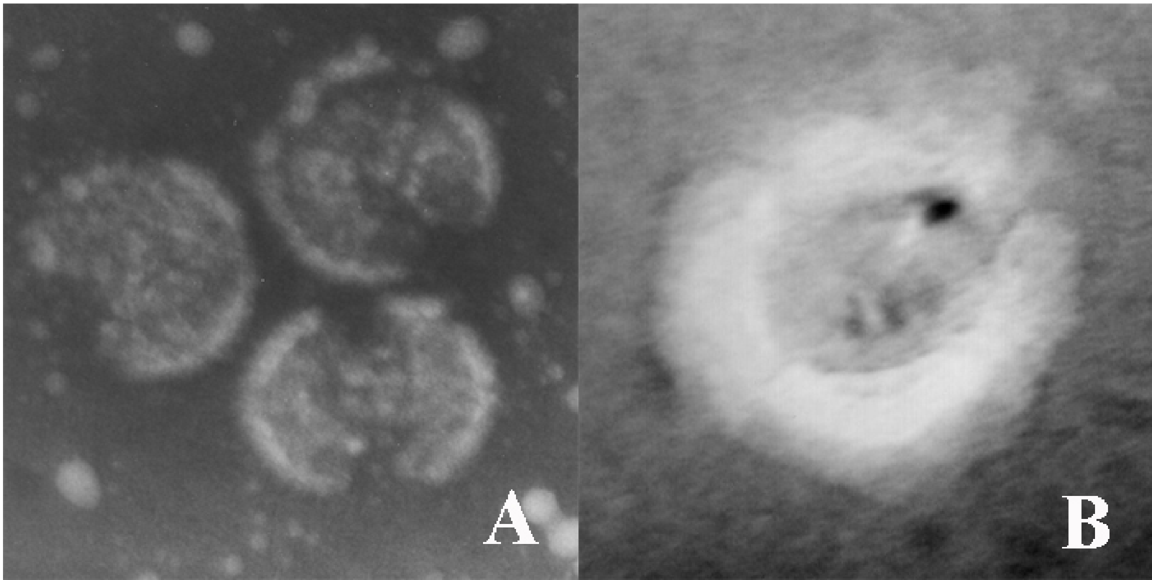


FIGURE 6. Transmission electron micrograph analysis of adenovirus type-2 in 100% DPBS, treated with 1200 ppmv ozone for 60 min. Ad2 was treated with 1200 ppmv ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. Samples were negatively stained with PTA and micrographs were taken at: (A) 110,000X and 120 kV; and (B) 110,000X, 120kV and enlarged with Adobe Photoshop. A broken protein capsid can be seen in (A) and Ad2 morphology appears altered in (B).

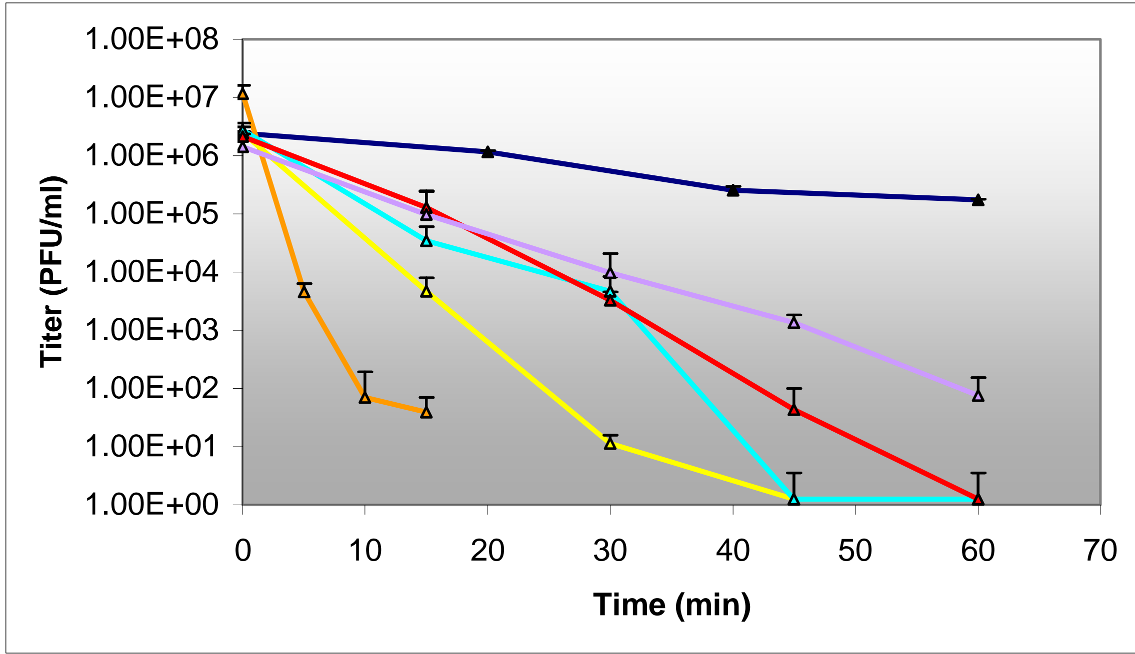


FIGURE 7. Inactivation of herpes simplex virus type-1 when treated with 1200 ppmv ozone. HSV-1 was treated under the conditions of oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. (▲) HSV-1 suspended in 100% DPBS with O_2 gas only. Points represent the averages of four replicates in one ozone treatment run. For the following, data points represent the averages of four replicates in two separate ozone treatment runs. (▲) HSV-1 suspended in 100% DPBS treated with 1200 ppmv ozone. (▲) HSV-1 suspended in 10% CCS in DPBS with 1200 ppmv ozone. (▲) HSV-1 suspended in 50% CCS in DPBS with 1200 ppmv ozone. (▲) HSV-1 suspended in 80% CCS in DPBS with 1200 ppmv ozone. (▲) HSV-1 suspended in 100% CCS treated with 1200 ppmv ozone. All y-axis error bars represent one standard deviation from the mean.

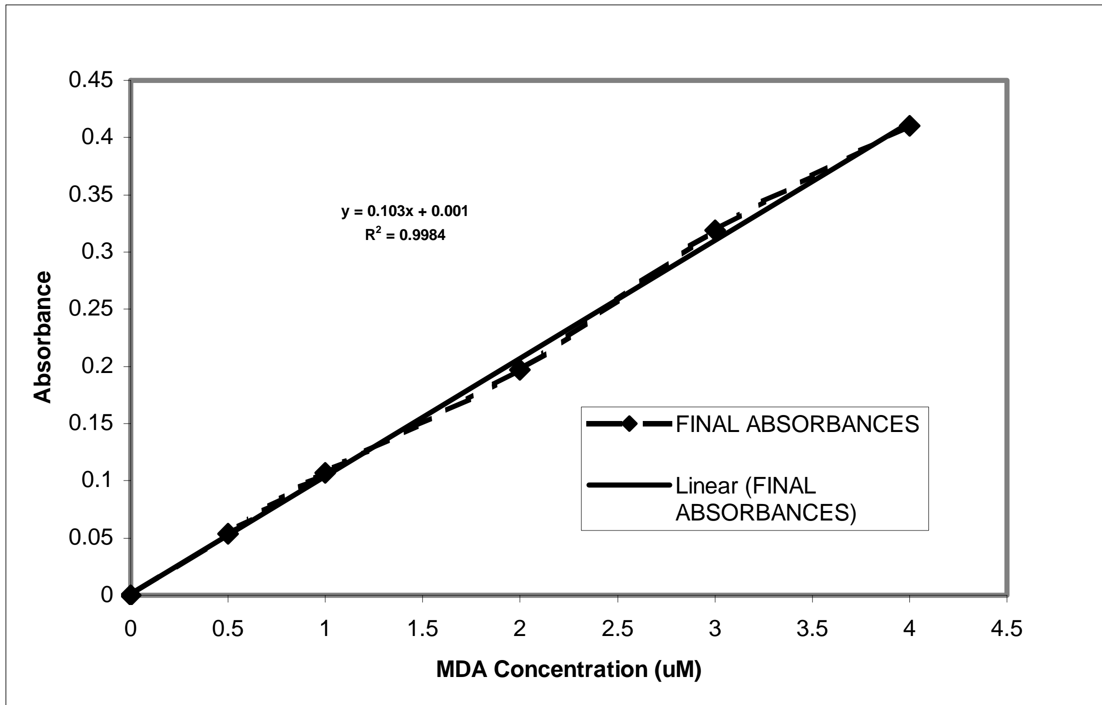


FIGURE 8. Standard curve of lipid peroxidation assay for herpes simplex virus type-1. This standard curve was used to derive the MDA concentrations found in ozone conditioned HSV-1 suspended in 100% DPBS (Fig. 9)**.**

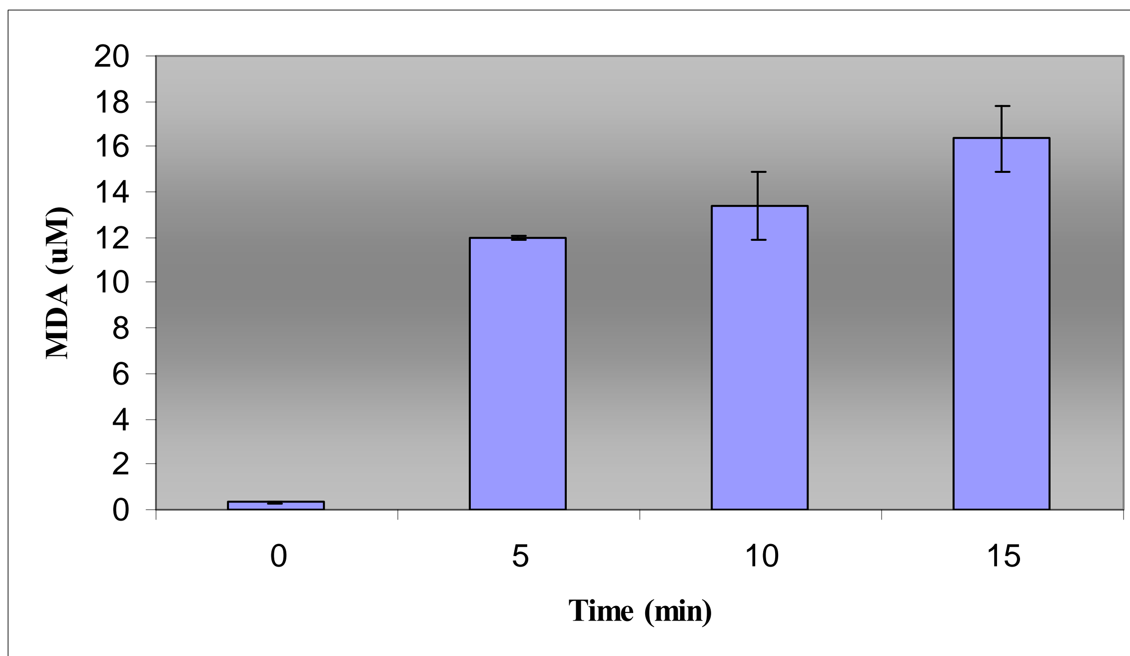


FIGURE 9. MDA concentrations for herpes simplex virus type-1 suspended in 100% DPBS, and treated with 1200 ppmv ozone. HSV-1 was suspended in 100% DPBS, and treated under the conditions of 1200 ppmv of ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. 0 min, 5 min, 10 min and 15 min ozone treatment samples were assayed for MDA content and reported in µM. Data are representative of two replicates runs from the same standard curve (Fig. 8). Error bars represent one standard deviation from the mean.

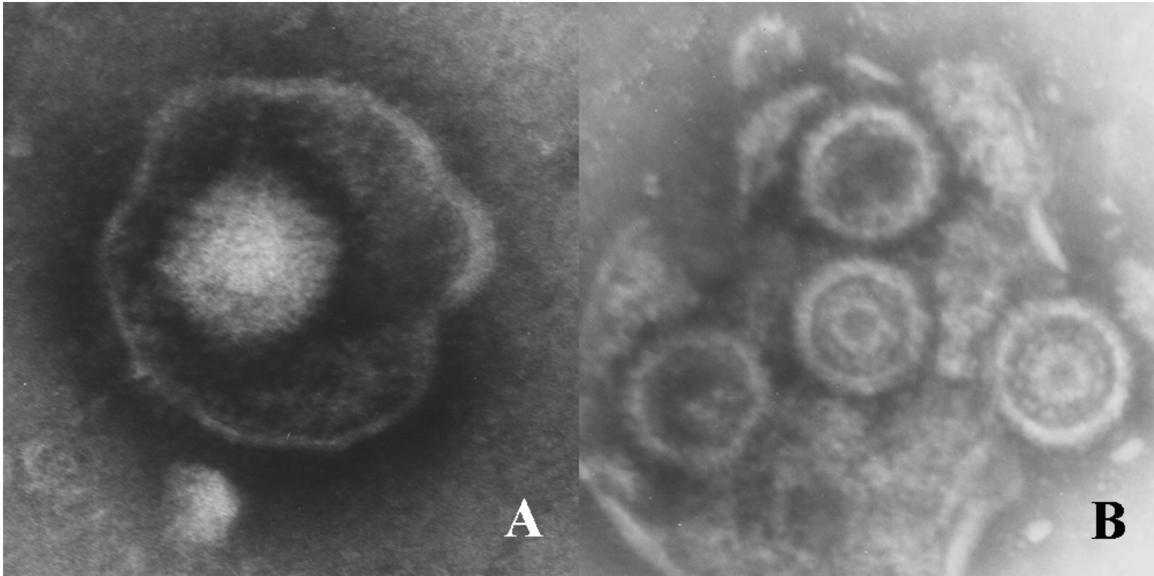


FIGURE 10. Transmission electron micrograph analysis of untreated herpes simplex virus type-1 suspended in 100% DPBS (1). Untreated HSV-1 was negatively stained with PTA and micrographs were taken at (A) 110,000X, 120 kV and enlarged with Adobe Photoshop; (B) 150,000X and 120 kV. An HSV-1 virion with an intact envelope is seen in (A) and an aggregate of HSV-1 particles is seen in (B).

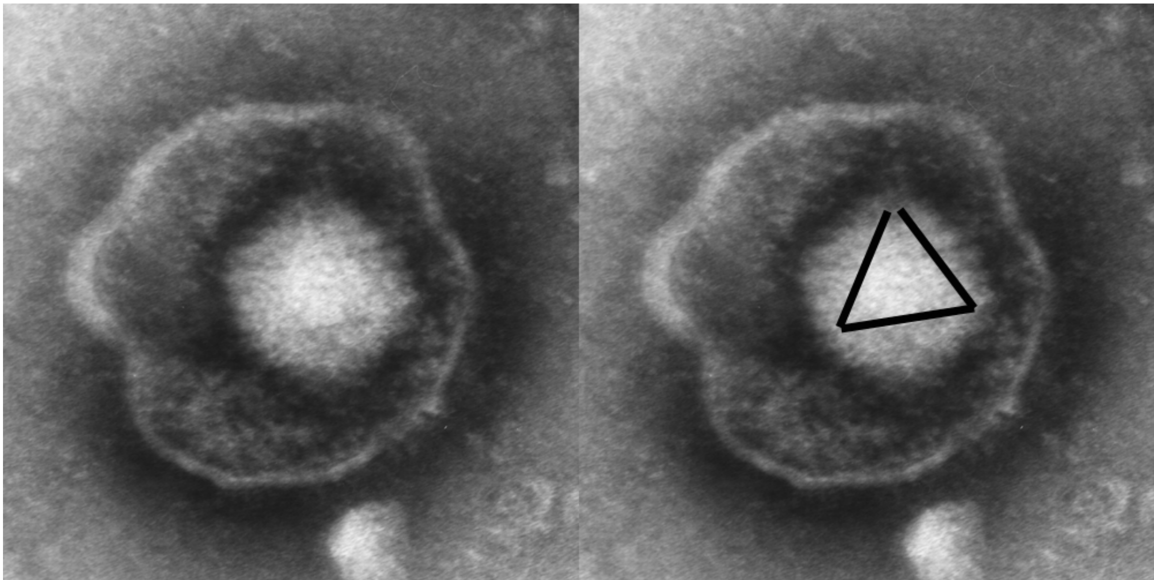


FIGURE 11. Transmission electron micrograph analysis of untreated herpes simplex virus type-1 suspended in 100% DPBS (2). Untreated HSV-1 was negatively stained with PTA and micrographs were taken at 150,000X, 120 kV and enlarged with Adobe Photoshop. The three-fold axis of symmetry is seen in (A) and is outlined in (B)

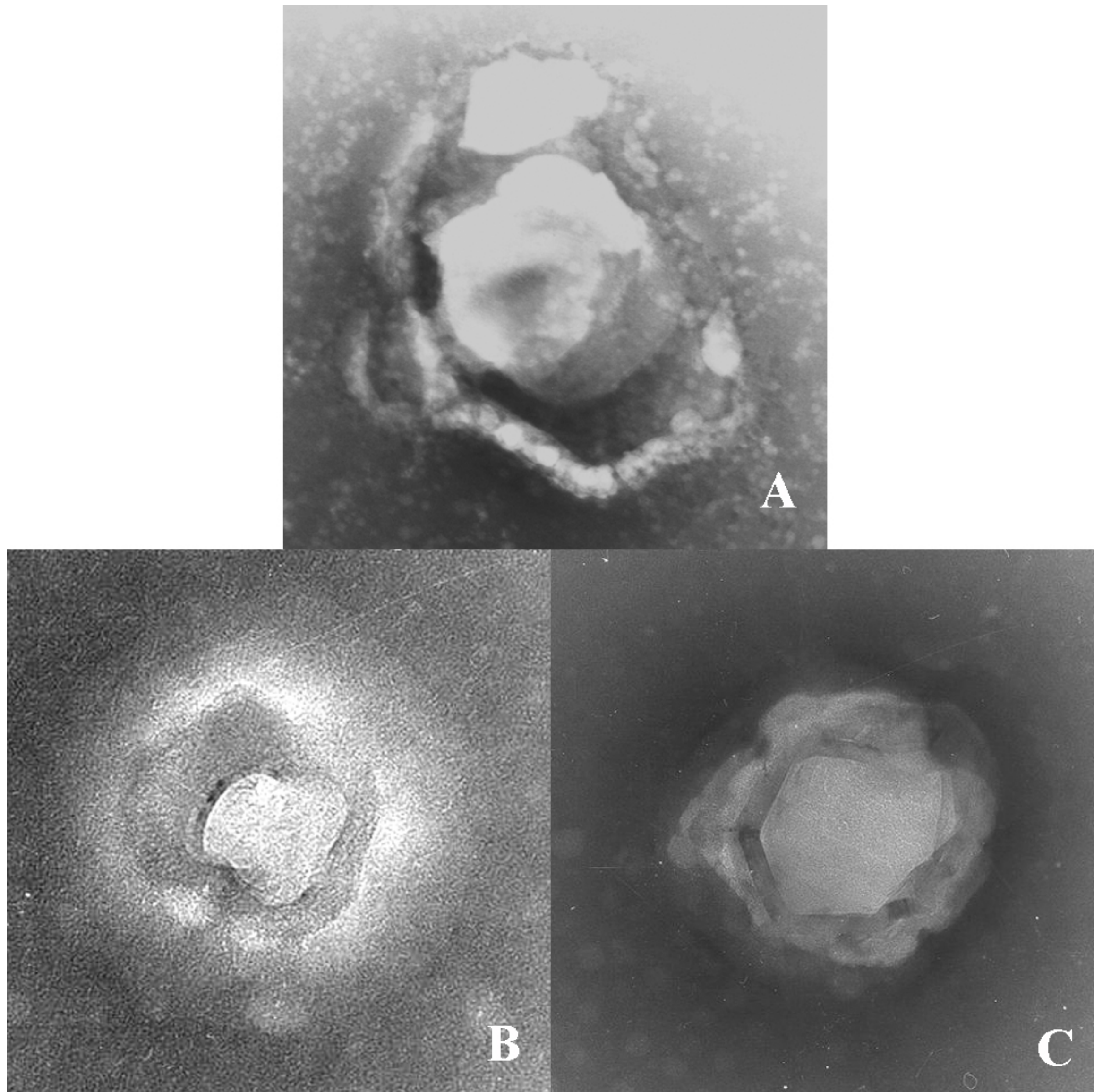


FIGURE 12. Transmission electron micrograph analysis of herpes simplex virus type-1 in 100% DPBS, treated with 1200 ppmv ozone for 15 min. HSV-1 was treated with 1200 ppmv ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. Samples were negatively stained with PTA and micrographs were taken at 52,000X, 120 kV and enlarged with Adobe Photoshop. The disrupted morphology of the icosehedral nucleocapsid is seen in (A, B, and C).

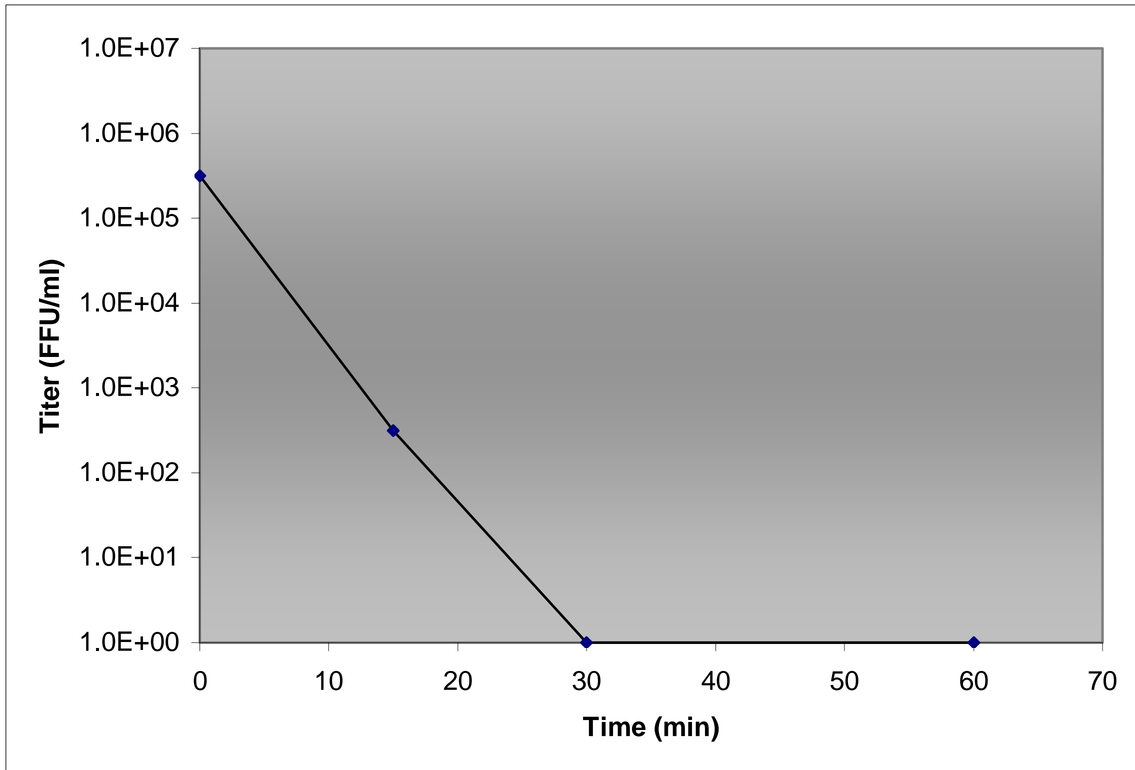


FIGURE 13. Inactivation of influenza A in DME with 0.125% BSA when treated with 1200 ppmv ozone. Influenza A was suspended in DME with 0.125% BSA, treated under conditions of 1200 ppmv of ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. Results shown are from one ozone treatment run, assayed in the laboratory of Dr. F.B. Johsnson.

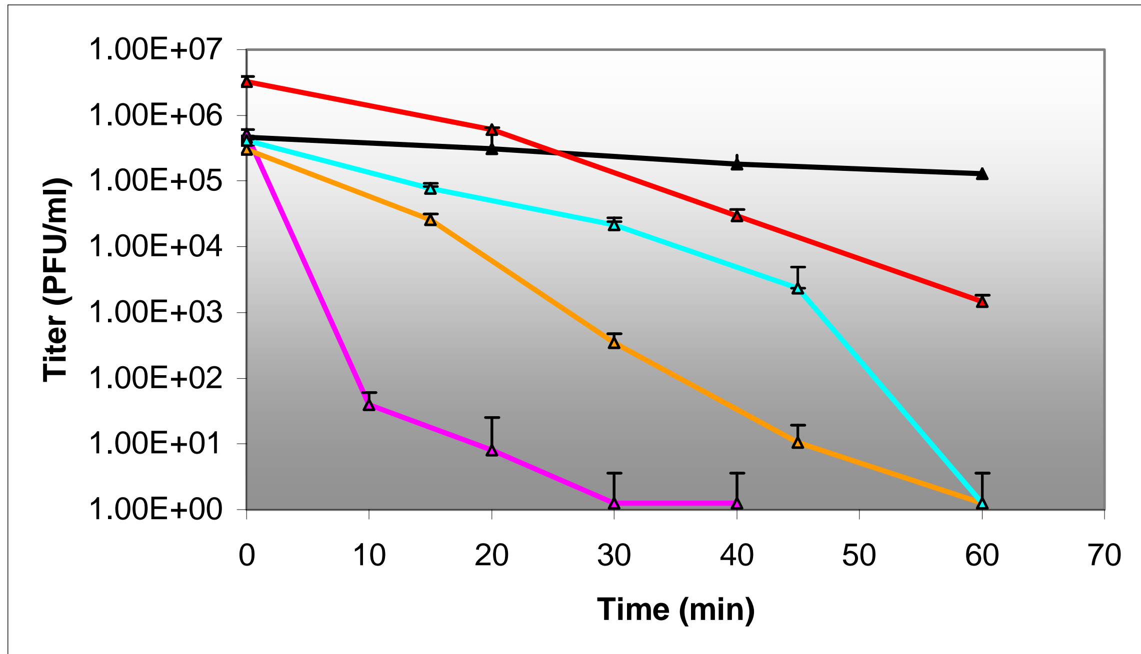


FIGURE 14. Inactivation of vaccinia virus when treated with 1200 ppmv ozone. VAC was treated under the conditions of oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. (▲) VAC suspended in 100% DPBS with O_2 gas only. Points represent the averages of four replicates in one ozone treatment run. For the following, data points represent the averages of four replicates in two separate ozone treatment runs. (▲) VAC suspended in 100% DPBS treated with 1200 ppmv ozone. (▲) VAC suspended in 10% CCS in DPBS with 1200 ppmv ozone. (▲) VAC suspended in 50% CCS in DPBS with 1200 ppmv ozone. (▲) VSV suspended in 100% CCS treated with 1200 ppmv ozone. All y-axis error bars represent one standard deviation from the mean.

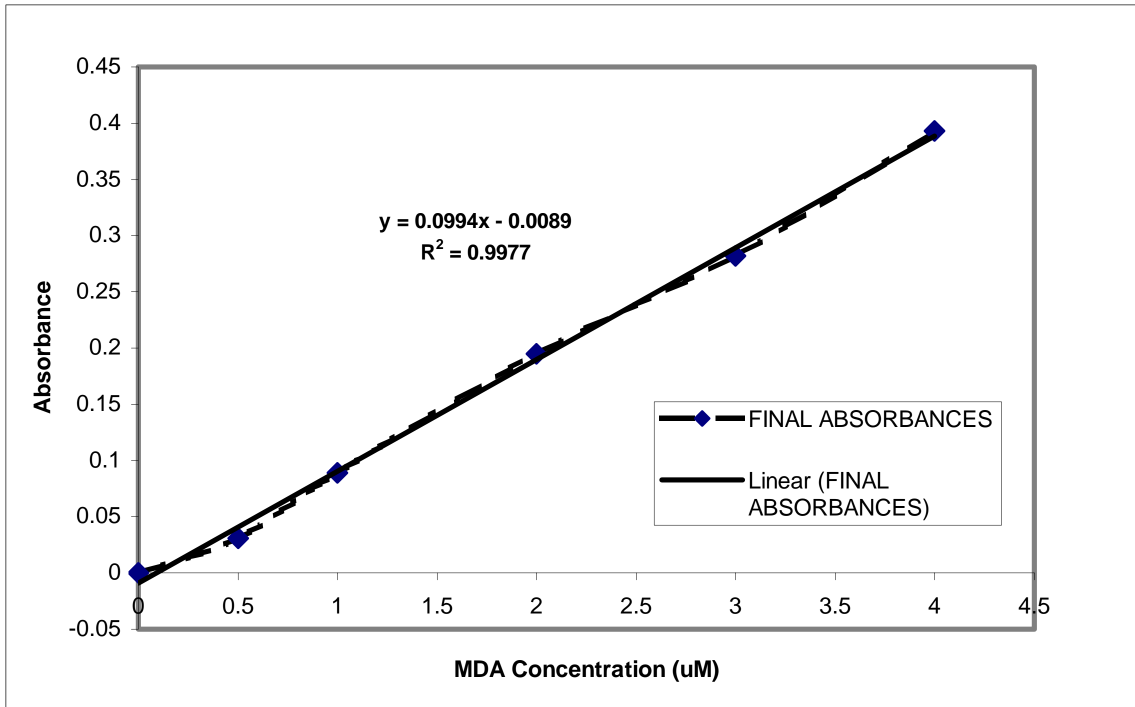


FIGURE 15. Standard curve of lipid peroxidation assay for vaccinia. This standard curve was used to derive the MDA concentrations found in ozone conditioned VAC suspended in 100% DPBS (Fig. 16)**.**

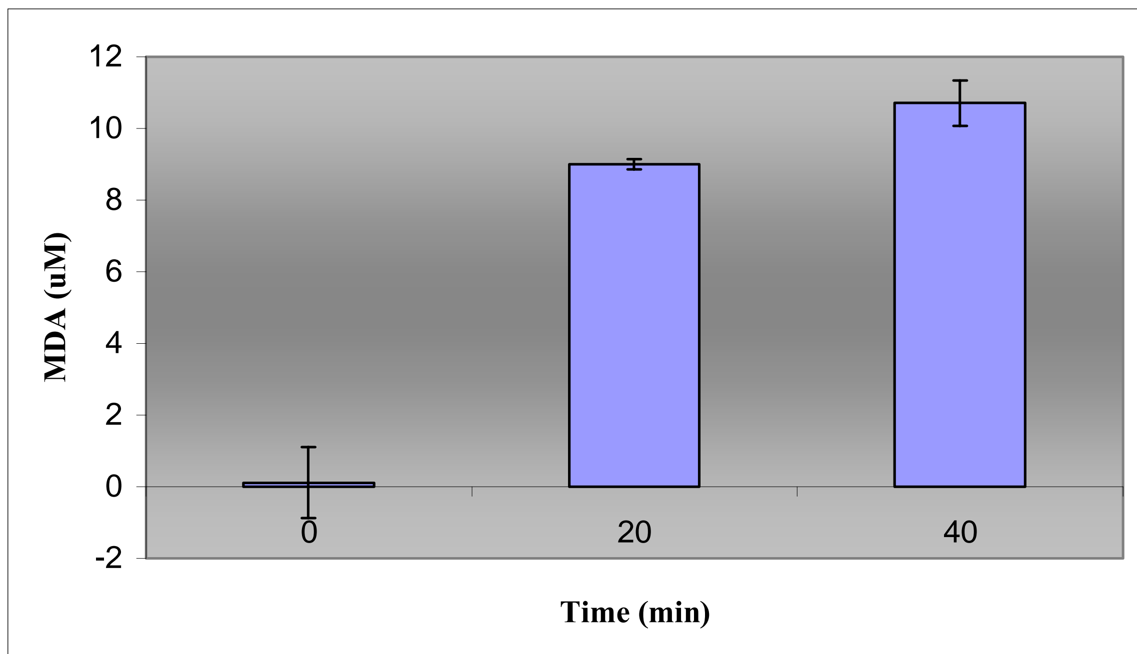
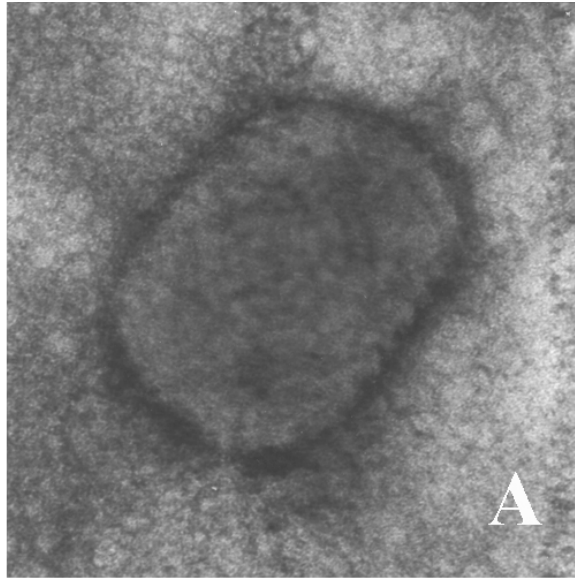
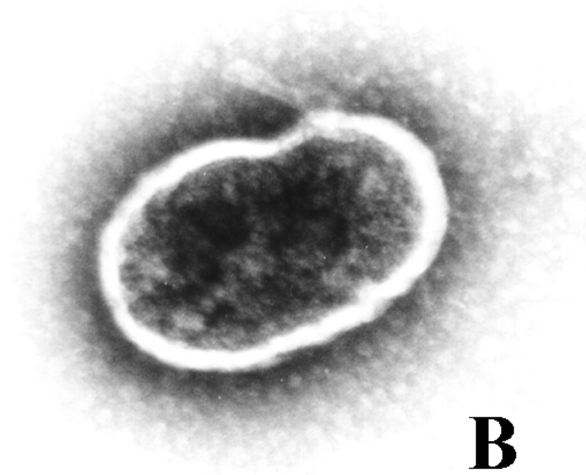


FIGURE 16. MDA concentrations for vaccinia virus suspended in 100% DPBS, and treated with 1200 ppmv ozone. VAC was suspended in 100% DPBS, and treated under the conditions of 1200 ppmv of ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. 0 min, 20 min, and 40 min ozone treatment samples were assayed for MDA content and reported in μM. Data are representative of two replicates runs from the same standard curve (Fig. 15). Error bars represent one standard deviation from the mean.





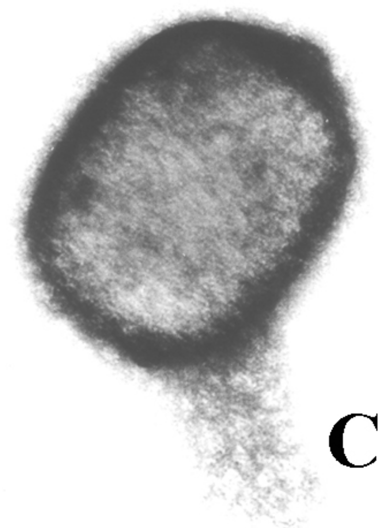


FIGURE 17. Transmission electron micrograph analysis of untreated vaccinia virus suspended in 100% DPBS. Untreated VAC was negatively stained with PTA and micrographs were taken at 52,000X, 120 kV and enlarged with Adobe Photoshop. VAC virions with familiar "rounded-rectangle" morphology are seen in (A, B and C)

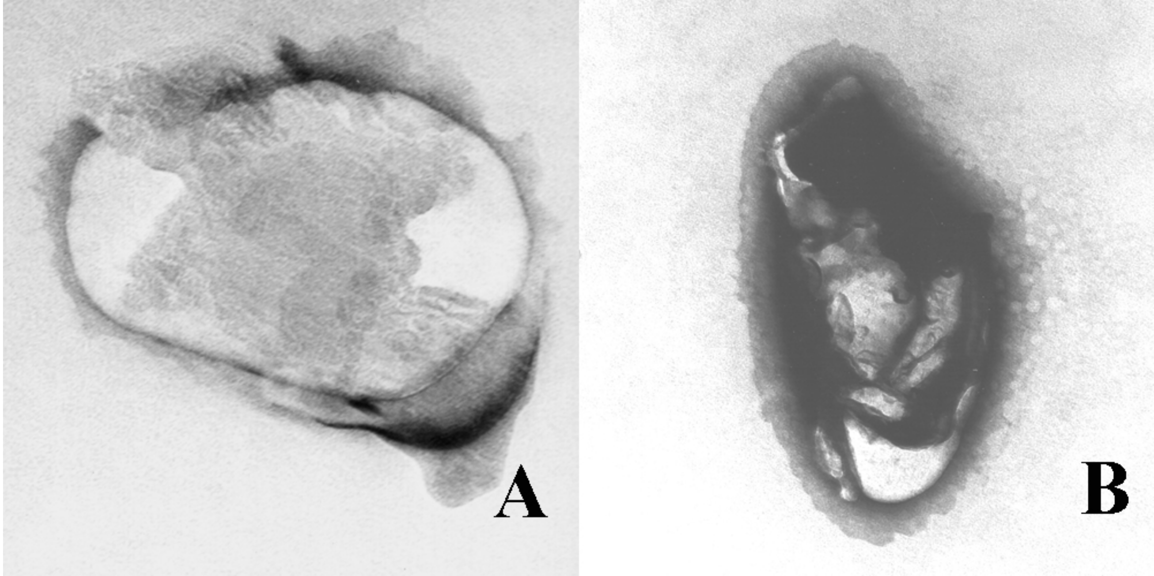


FIGURE 18. Transmission electron micrograph analysis of vaccinia virus in 100% DPBS, treated with 1200 ppmv ozone for 40 min. VAC was treated with 1200 ppmv ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. Samples were negatively stained with PTA and micrographs were taken at 42,000X, 120 kV and enlarged with Adobe Photoshop. The disrupted morphology of the VAC is seen in (A and B). Extensive internal damage of the VAC particle can be seen in (B).

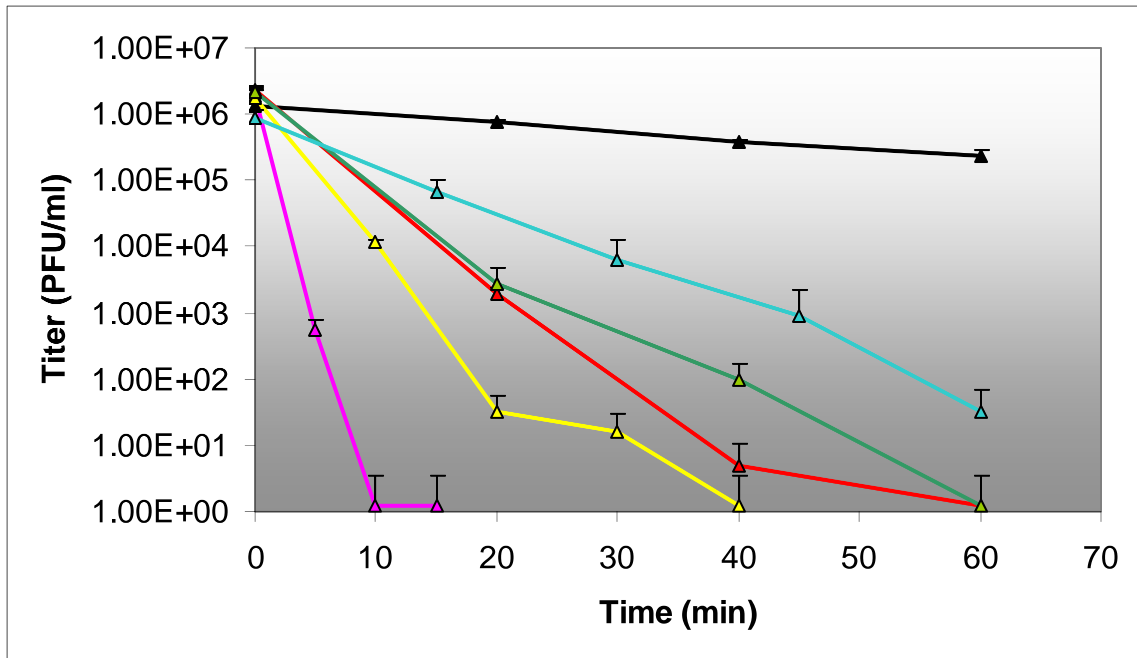


FIGURE 19. Inactivation of vesicular stomatitis virus when treated with 1200 ppmv ozone. VSV was treated under the conditions of oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. (▲) VSV suspended in 100% DPBS with O_2 gas only. Points represent the averages of four replicates in one ozone treatment run. For the following, data points represent the averages of four replicates in two separate ozone treatment runs. (▲) VSV suspended in 100% DPBS treated with 1200 ppmv ozone. (▲) VSV suspended in 10% CCS in DPBS with 1200 ppmv ozone. (▲) VSV suspended in 50% CCS in DPBS with 1200 ppmv ozone. (▲) VSV suspended in 80% CCS in DPBS with 1200 ppmv ozone. (▲) VSV suspended in 100% CCS treated with 1200 ppmv ozone. All y-axis error bars represent one standard deviation from the mean.

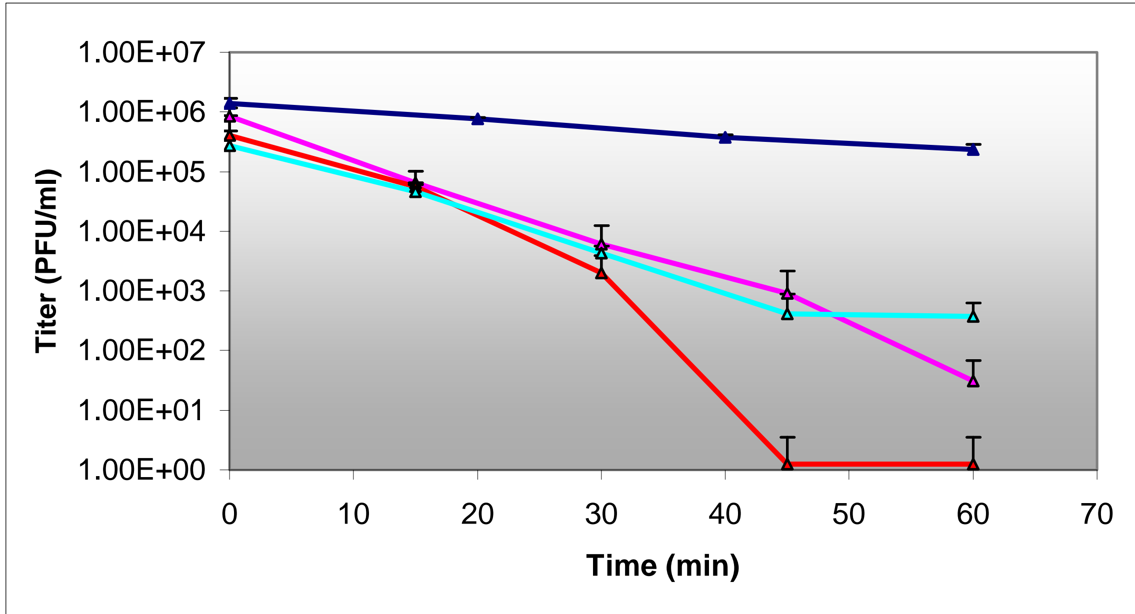


FIGURE 20. Inactivation of vesicular stomatitis virus suspended in 100% CCS and treated with various ppmv of ozone. VSV was treated under the conditions of oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. (▲) VSV suspended in 100% DPBS with O_2 gas only. Points represent the averages of four replicates in one ozone treatment run. For the following, data points represent the averages of four replicates in two separate ozone treatment runs. (▲) VSV suspended in 100% CCS treated with 1500 ppmv ozone. Data points represent the averages of four replicates from one ozone treatment run. (▲) VSV suspended in 100% CCS with 1200 ppmv ozone. Data points represent the averages of four replicates in two separate ozone treatment runs (▲) VSV suspended in 100% CCS with 1500 ppmv ozone. Data points represent the averages of four replicates from one ozone treatment run. All y-axis error bars represent one standard deviation from the mean.

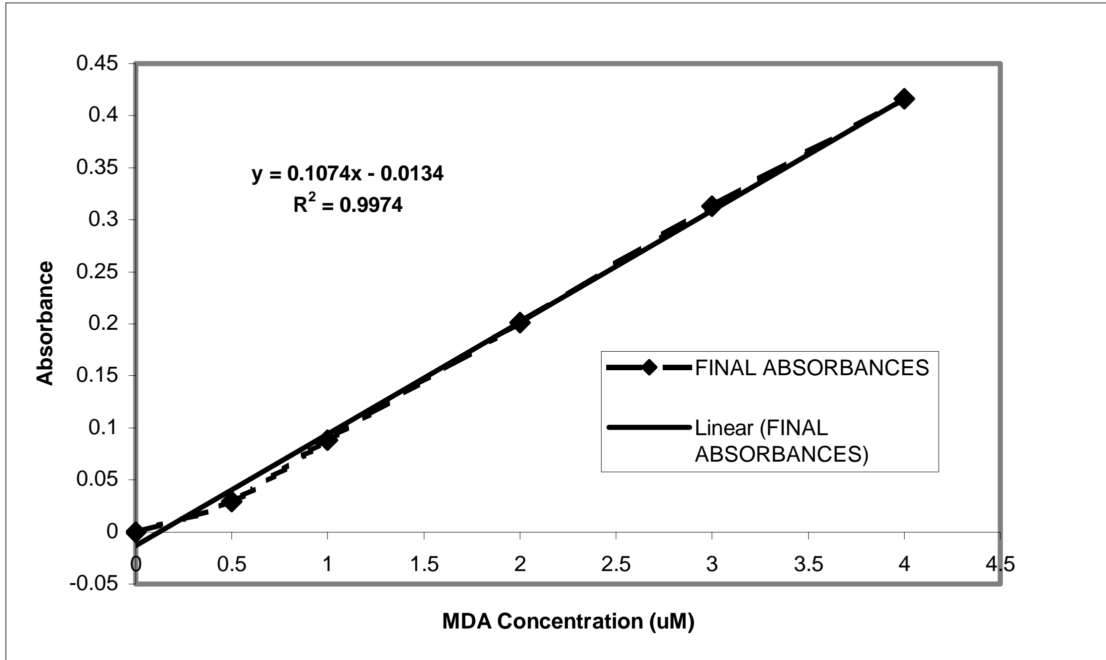


FIGURE 21. Standard curve of lipid peroxidation assay for vesicular stomatits virus. This standard curve was used to derive the MDA concentrations found in ozone conditioned VSV suspended in 100% DPBS (Fig. 22)**.**

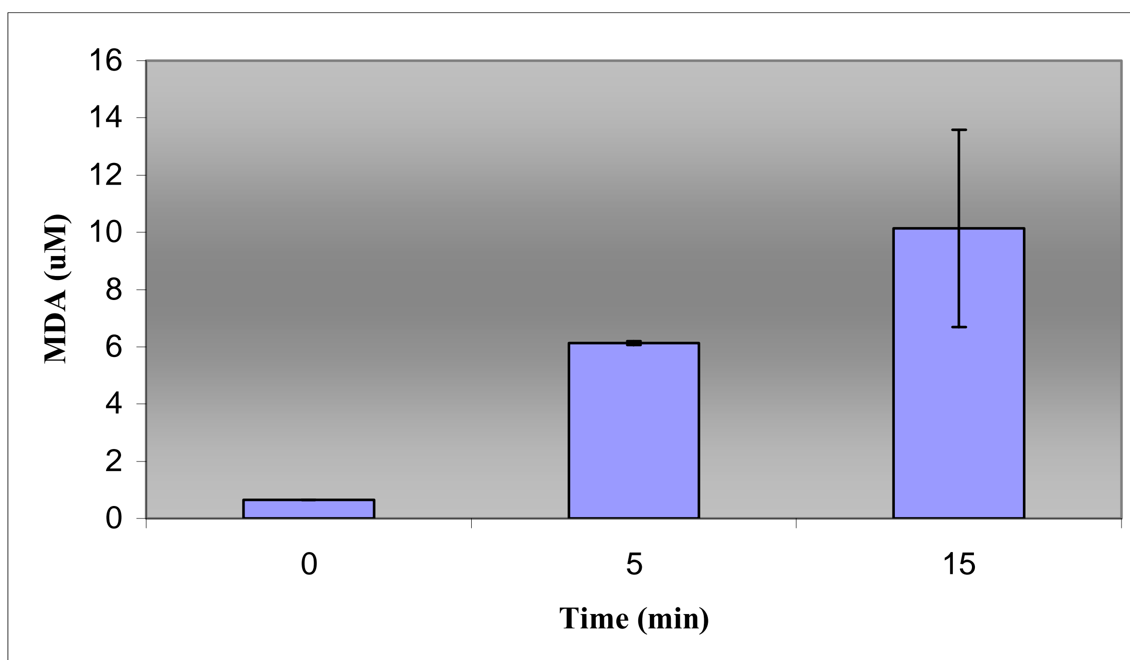


FIGURE 22. MDA concentrations for vesicular stomatitis virus suspended in 100% DPBS, and treated with 1200 ppmv ozone. VSV was suspended in 100% DPBS, treated under conditions of 1200 ppmv of ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. 0, 5 and 15 min ozone treatment samples are reported and MDA concentrations are reported as µM. Data are representative of two replicates from the same standard curve (Fig. 21). Error bars represent one standard deviation from the mean.

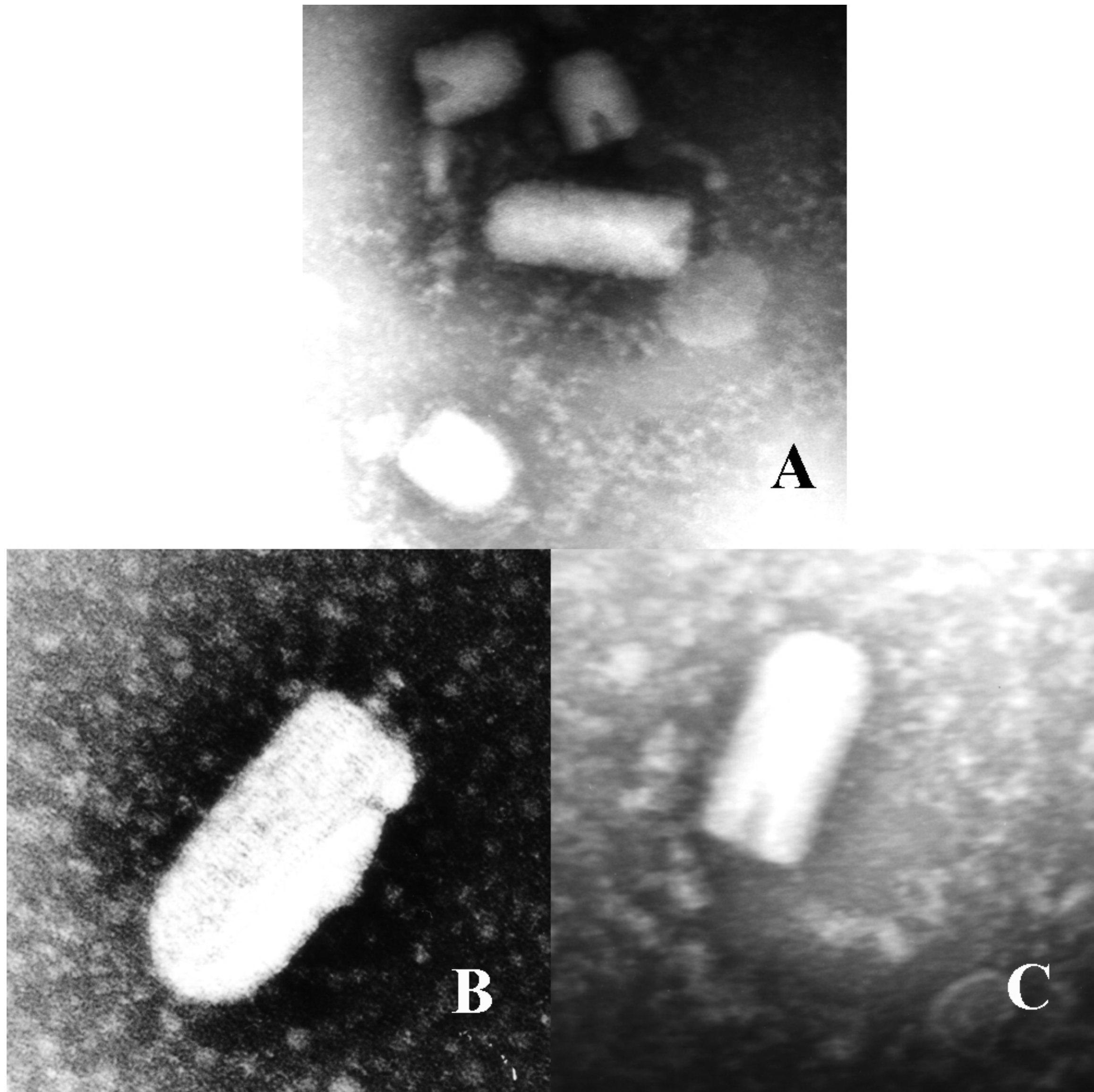


FIGURE 23. Transmission electron micrograph analysis of untreated vesicular stomatitis virus suspended in 100% DPBS. Untreated VSV was negatively stained with PTA and micrographs were taken at: (A) 150,000X and 120 kV, (B) 150,000X, 120kV and enlarged with Adobe Photoshop, and (C) 150,000X, 120kV and enlarged with Adobe Photoshop. VSV virions with familiar “bullet-shaped” morphology are seen in (A, B and C).

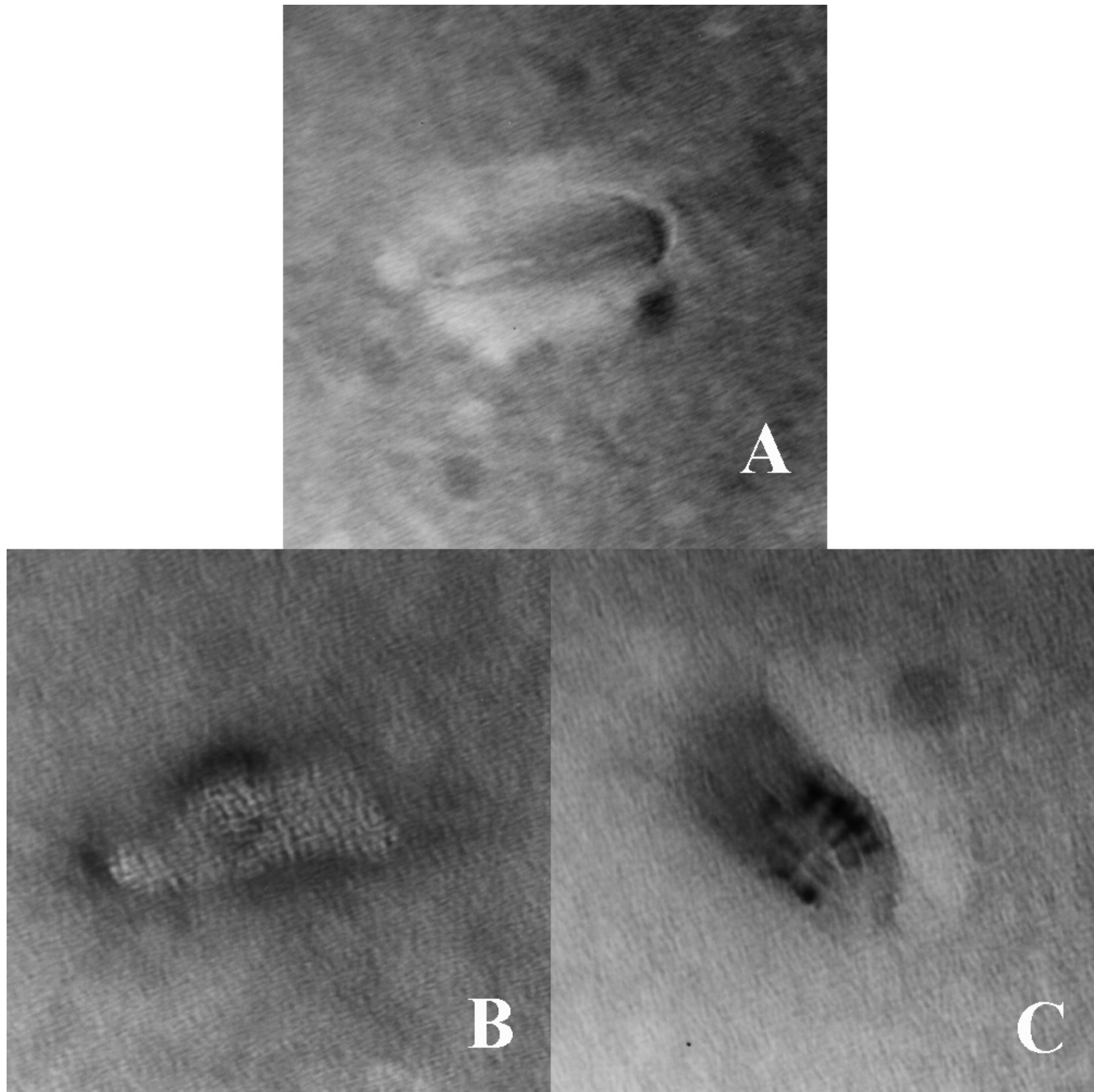


FIGURE 24. Transmission electron micrograph analysis of vesicular stomatitis virus in 100% DPBS, treated with 1200 ppmv ozone for 15 min. VSV was treated with 1200 ppmv ozone, oxygen flow rate of 2000 ml/min and fluid pump rate of 252 ml/min. Samples were negatively stained with PTA and micrographs were taken at: (A and B) 220,000X and 120 kV, and (C) 250,000X and 120 kV. The disrupted morphology of VSV is seen in (A, B and C).

TABLE 2. Summary of ozone-mediated viral inactivation data.

Virus	Max[a]	Time (min)[b]	Min[c]	Time (min)[d]	MDA concentration (μM)[e]	TEM[f]
Adenovirus type-2	5-$\log_{10}$	60	3-$\log_{10}$	60	20.27	+
Herpes simplex virus type-1	6-$\log_{10}$	15	4.5-$\log_{10}$	60	16.03	+
Influenza A[g]	5-$\log_{10}$	30				
Vaccinia virus	5-$\log_{10}$	30	3-$\log_{10}$	60	10.59	+
Vesicular stomatits virus	6-$\log_{10}$	10	3-$\log_{10}$	60	9.49	+

[a] – Maximum virus inactivation for corresponding species, virus suspended in 100% DPBS, 1200 ppmv of ozone.
[b] – Ozone treatment time for maximum inactivation.
[c] – Minimun virus inactivation for corresponding species, virus suspended in 100% CCS, 1200 ppmv of ozone.
[d] – Ozone treatment time for minimum inactivation.
[e] – Change in MDA concentration for maximum virus inactivation series.
[f] – Detection of morphological changes of corresponding virus through transmission electron microscopy image analysis.
[g] – Influenza virus was performed under one condition only, no MDA and TEM data.

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Scientific presentations during my research at the Microbiology and Molecular Biology program at Brigham Young University*:*

Published articles:

- *S.G. Aldana, R.L. Greenlaw, H.A. Diehl, A. Salsberg, R.M. Merrill,* ***S. Ohmine****, C. Thomas, and J. Olsen.* The effects of an intensive diet and physical activity modification program on the health risks of adults. *J Am Diet Assoc*. 2005 Mar; 105(3):371-81.

Submitted for Publication:

- *S.G. Aldana, R.L. Greenlaw, H.A. Diehl, A. Salsberg, R.M. Merrill,* ***S. Ohmine*** *and C. Thomas.* The behavioral and clinical effects of therapeutic lifestyle change on middle-aged adults. Submitted for publication December 2004 in the Archives of Internal Medicine.
- *S.G. Aldana, R.L. Greenlaw, H.A. Diehl, A. Salsberg, R.M. Merrill and* ***S. Ohmine.*** The effects of a worksite chronic disease prevention program. Submitted for publication December 2004 in the Journal of Occupational and Environmental Medicine.

Manuscripts Currently in Process:

- ***S. Ohmine****, D.P. Tomer, L.D. McLeman, K.L. O'Neill and B.K. Murray*. A reliable and rapid method for quantification of C-reactive protein in a latex bead-enhanced immunoturbidimetric assay. Submitting to Biotechniques.
- ***S. Ohmine****, D.P. Tomer, K.L. O'Neill, and B.K. Murray*. Total antioxidant activites of *Daizu* (soybean)-based foods found in the Japanese diet. Submitting to Journal of Food and Agricultural Chemistry.
- *D.P. Tomer, L.D. McLeman,* ***S. Ohmine****, B.K. Murray and K.L. O'Neill.* Correlation between the TOSC and ORAC antioxidant assays for measuring the antioxidant activity of phytochemicals. Submitting to Free Radical Biology and Medicine.

Poster Presentations

- ***S.Ohmine***, *M.A. Viskovska, D.P. Tomer, S.B. Stringham, J.S. Latino, K.L. O'Neill, F. B. Johnson and B.K. Murray*. Loss of herpes simplex virus type-1 infectivity by reactive oxygen species – *presented at the American Society for Microbiology 105th General Meeting; 2005/06/5-9*.
- ***S.Ohmine****, D.P. Tomer, N.J. Buchkovich, J.S. Latino, K.L. O'Neill, F.B. Johnson and B.K. Murray.* Reactive oxygen species inactivation of enveloped and non-enveloped viruses – *presented at the American Society for Microbiology 105th General Meeting; 2005/06/5-9*.
- ***S. Ohmine****, C.R. Trimble, D.P. Tomer, L.D. McLeman, K.L O'Neill and B.K. Murray.* Ameliorization of oxidative stress in human leukemia cells by antioxidant synergism – *presented at the American Association for Cancer Research 96th Annual Meeting; 2005/04/16-20.*
- ***S. Ohmine****, L.D. McLeman, D.P. Tomer, K.L. O'Neill and B.K. Murray.* Stress-mediated induction of C-reactive protein in human cancer cells – *presented at the American Association for Cancer Research 95th Annual Meeting; 2004/03/27-31.*

- ***S. Ohmine**, K.L. O'Neill, and B.K. Murray.* Total Oxiradical Scavenging Capacity of *Daizu* (soybean)-based foods in the asian diet. – *presented at the American Association for the Advancement of Science Annual Meeting; 2003/02/15-18, Denver, Colorado.*
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- *D.D. Twitchell, **S. Ohmine**, T.A. Gaufin, R.L. Hamblin, K.L. O'Neill and B.K. Murray.* Stromal cell derived factor 1α and macrophages increased invasive potential of breast cancer cells expressing CXCR4 – *presented at the American Association for Cancer Research 96th Annual Meeting; 2005/04/16-20.*
- *C.R. Trimble, **S. Ohmine**, T.R. Hart, T.A. Gaufin, M.A. Viskovska, R.A. Robison, K.L. O'Neill and B.K. Murray.* Membrane fatty acid composition in cancer cells: implications for chemotherapeutic intervention – *presented at the American Association for Cancer Research 96th Annual Meeting; 2005/04/16-20.*
- *L.D. McLeman, **S. Ohmine**, D.P. Tomer, M. Phillips, B. Webb, K.L. O'Neill and B.K. Murray.* Evidence of synergistic intracellular antioxidant networking – *presented at the American Association for Cancer Research 95th Annual Meeting; 2004/03/27-31.*
- *D.D. Twitchell, T. A. Gaufin, **S.Ohmine**, R.L. Hamblin, K. L. O'Neill and B.K. Murray* A model for enhanced cancer cell invasion by stromal cell derived factor-1α and macrophages – *presented at the 8th Cancer Research UK Beatson International Cancer Conference; 2005/06/19-22.*
- *S.B. Stringham, B.K. Murray, K.L. O'Neill, **S. Ohmine**, T.A. Gaufin and W.G. Pitt.* Mechanism of targeted chemotherapeutic delivery using ultrasound – *presented at the American Association for Cancer Research 96th Annual Meeting; 2005/04/16-20.*

Oral Presentations

- ***S.Ohmine**, D.P. Tomer, N.J. Buchkovich, J.S. Latino, K.L. O'Neill, F.B. Johnson and B.K. Murray.* Inactivation of Scrapie Prion Infectivity by Reactive Oxygen Species – *presented at the International Union of Microbiological Societies General Assembly Meeting; 2005/07/23-28.*
- *T.A. Gaufin, **S.Ohmine,** M.A. Viskovska, D.P. Tomer, N.J. Buchkovich, J.S. Latino, K.L. O'Neill, F.B. Johnson and B.K. Murray.* Reactive Oxygen Species-Mediated Loss of Vesicular Stomatitis Virus Infectivity – *presented at the International Union of Microbiological Societies General Assembly Meeting; 2005/07/23-28.*
- ***S.Ohmine**, O. Badamjav, D.P. Tomer, L.D. McLeman, K.L. O'Neill and B.K. Murray.* Reduction of oxidative stress in human leukemia cells by combinational antioxidant synergism – *awarded first place at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*
- ***S.Ohmine**, N.J. Buchkovich, T.A. Gaufin, M.P. Gutiérrez, D.P. Tomer, L.D. McLeman, S.B. Stringham, C.Trimble, K. Hamik, K.L. O'Neill and B.K. Murray.* Parameters involved in maintaining biological integrity in fluids following ozone exposure. – *presented at the American Society for Microbiology Intermountain Branch Meeting; Idaho Falls, Idaho 2004/03/27.*

- ***S.Ohmine**, T.A. Gaufin, M.P. Gutiérrez, L.D. McLeman, D.P. Tomer, K. Hamik, F.B. Johnson, K.L. O'Neill and B.K. Murray.* Inactivation of animal viruses in biological fluids following ozone exposure. *– presented at the American Society for Microbiology Intermountain Branch Meeting; Idaho Falls, Idaho 2004/03/27.*
- ***S.Ohmine**, M.P. Gutiérrez, T.A. Gaufin, L.D. McLeman, D.P. Tomer, K.L. O'Neill and B.K. Murray.* Development of a regulated cell culture C-reactive protein biomarker assay *– presented at the American Society for Microbiology Intermountain Branch Meeting Conference; Idaho Falls, Idaho 2004/03/27.*
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- *T.A. Gaufin, **S. Ohmine**, M.A. Viskovska, D.P. Tomer, N.J. Buchkovich, J.S.Latino, K.L. O'Neill, F.B. Johnson and B.K. Murray*. Reactive oxygen species-mediated loss of vesicular stomatitis virus infectivity – presenting at the IUMS Joint meeting; 2005/07/23-28.
- *L.D. McLeman, **S. Ohmine**, D.P. Tomer, S.G. Aldana, K.L. O'Neill, and B.K. Murray.* A Comparitive analysis of Total Antioxidant Capacity in Various Body Fluids as Measured by the Fluorometric Oxyradical Absorbance Capacity (ORAC) Assay. *– presented at the American Society for Microbiology Intermountain Branch Meeting Tooele, UT 2003/03/29.*
- *M.A. Viskovska, D.C. Harrell, **S. Ohmine**, T.A. Gaufin, D.P. Tomer, C.R. Trimble, N.J. Buchkovich, J. Latino, K.L. O'Neill, F.B. Johnson, and B.K. Murray.* Loss of Vesicular Stomatitis Virus Infectivity Through Ozone-Generated Reactive Oxygen Species *– presented at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*
- *T.A. Gaufin, K.J. Jensen, **S. Ohmine**, M.A. Viskovska, D.P. Tomer, N.J. Buchkovich, S.B. Stringham, J. Latino, K.L. O'Neill, F.B. Johnson, and B.K. Murray.* Herpes Simplex Virus Type-1 Inactivation Through Ozone-Mediated Peroxidation *– presented at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*
- *T.A. Gaufin, J.W. Monson, **S. Ohmine**, M.A. Viskovska, D.P. Tomer, N.J. Buchkovich, J. Latino, K.L. O'Neill, F.B. Johnson, and B.K. Murray.* Inactivation Kinetics of Enveloped and Non-Enveloped Viruses Through Reactive Oxygen Species (ROS) *– presented at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*
- *M.A. Viskovska, T.A. Gaufin, **S. Ohmine**, D.D. Twitchell, K.L. O'Neill and B.K. Murray.* Increased Invasive Potential of CXCR4+ Breast Cancer Cells in the Presence of Stromal Cell Derived Factor-1α and Macrophages *– presented at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*
- *D.P. Tomer, L.D. McLeman, **S. Ohmine**, S.B. Stringham, C. Trimble, N.J. Buchkovich, K.L. O'Neill, and B.K. Murray.* Lipid Peroxides Generated by the Ozonation of Biological Fluids are Effectively Neutralized by Phytochemicals with Antioxidant Capacity. *– presented at the American Association for the Advancement of Science Pacific Meeting; Logan, UT 2004/06.*

- *D.P. Tomer, L.D. McLeman, **S.Ohmine**, S.B. Stringham, C.Trimble, N.J. Buchkovich, K. Hamik, K.L. O'Neill and B.K. Murray.* Correlation Between Antioxidant Activity of Phytochemicals and Ability to Quench Lipid Peroxides. *– presented at the American Society for Microbiology Intermountain Branch Meeting; Idaho Falls, Idaho 2004/03/27.*
- *C. Trimble, L.D. McLeman, **S.Ohmine**, S.B. Stringham, D.P. Tomer, K.L. O'Neill and B.K. Murray.* Measurement of MDA Levels as a Marker of Lipid Peroxidation in Serum. *– presented at the American Association for the Advancement of Science Pacific Meeting; Logan, UT 2004/06.*
- *C.Trimble, L.D. McLeman, **S.Ohmine**, S.B. Stringham, D.P. Tomer, K. Hamik, K.L. O'Neill and B.K. Murray.* Analysis of Lipid Peroxidation Levels in Serum Following Exposure to Different Concentrations and Time Periods of Ozone Treatment. *– presented at the American Society for Microbiology Intermountain Branch Meeting; Idaho Falls, Idaho 2004/03/27.*
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- *S.B. Stringham, B.K. Murray, K.L. O'Neill, **S. Ohmine**, T.A. Gaufin, and W.G. Pitt.* Role of Collapse Cavitation in Ultrasound Mediated Chemotherapeutic Drug Uptake *– presented at the American Society for Microbiology Intermountain Branch Meeting; 2005/03/12, Ogden, Utah.*

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EFFECT OF AN INTENSIVE DIET AND PHYSICAL ACTIVITY MODIFICATION PROGRAM ON THE HEALTH RISKS OF ADULTS.

THE BEHAVIORAL AND CLINICAL EFFECTS OF THERAPEUTIC LIFESTYLE CHANGE ON MIDDLE-AGED ADULTS

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ABSTRACT

BACKGROUND

This study determined the behavioral and clinical impact of a therapeutic lifestyle modification intervention on a group of community volunteers.

METHODS

Participants included 348 volunteers aged 24-81 years from the Rockford, IL, metropolitan area who participated in a randomized clinical trial. The intervention group attended a 40-hour lecture educational course delivered over a four-week period. Participants learned the importance of making better lifestyle choices and how to make improvements in nutrition and physical activity. Changes in nutrition and physical activity behavior, and several chronic disease risk factors, were assessed at baseline and 6 months.

RESULTS

Beneficial mean changes in scores tended to be significant for the intervention group but not for the control group. Intervention participants showed significant 6-month change in all nutrition and physical activity measures, and all clinical measures except triglycerides and high sensitivity C-reactive protein. Total cholesterol and LDL were slightly worse after six months. Change score comparisons between the intervention and control groups were significant for all nutrition and physical activity variables, weight, body fat, diastolic blood pressure, resting heart rate, and HDL. The control group experienced comparatively small but significant improvements in systolic and diastolic blood pressure, body fat, and HDL, but reported significantly worse total cholesterol and LDL.

CONCLUSIONS

This therapeutic lifestyle modification program can significantly improve nutrition and physical activity behavior and can reduce many of the risk factors associated with common chronic diseases.

INTRODUCTION

Chronic diseases such as cancer, cardiovascular disease, stroke, and diabetes are responsible for most deaths in the United States.[1] Between 70% and 90% of these deaths are believed to be caused by poor nutrition, sedentary living, and tobacco use and are preventable.[2-4] These lifestyle factors appear to play a prominent role in the mechanisms and processes that lead to the development of many of these chronic diseases. The largest reductions in chronic disease prevalence in the US will be achieved when individuals adopt and maintain lifestyles that include a healthy diet and regular physical activity.

The Coronary Health Improvement Project (CHIP) was created with the goal of reducing chronic diseases and improving the overall health of the public by providing a lifestyle change program to both the community and the workplace.[5] The CHIP program is a 40-hour live-lecture educational course that highlights the importance of making better lifestyle choices for reducing chronic disease risk factors. A one-group pre-test/post-test analysis of the program revealed that after four weeks participants were able to significantly reduce blood pressure, blood glucose, body weight, and total and LDL cholesterol.[5] This exploratory study demonstrated that the program had the potential to improve not only coronary risk factors, but also reduce the risks associated with cancer, diabetes, and the metabolic syndrome. These results were repeated in a quasi experimental design that included results from six different groups of working adults.[6]

A large randomized clinical trial was initiated to further explore the effect of the program. Six-week results from this study revealed that in the short term, adults who complete the program improve nutrition and physical activity behavior and reduce most

chronic disease risk factors.[7] The current paper presents the behavioral and clinical changes that participants in this therapeutic lifestyle change program experienced after six months.

METHODS

SUBJECT RECRUITMENT AND DESIGN

Recruitment was conducted by the SwedishAmerican Center for Complementary Medicine (SACCM) using targeted advertising, marketing through the Centers of Excellence, CHIP alumni groups, corporate client sites, and the SwedishAmerican Medical Group. Recruitment efforts were aimed at adults (at least 18 years of age) in the greater Rockford, IL, metropolitan area. To be enrolled in the study, each participant had to be willing to participate in the program starting in one month or in seven months. Figure 1 shows the participant progress through the study. Eligible and interested participants provided informed consent. Participants were highly encouraged to participate with a spouse or significant other and were randomized as a paired unit. All other participants were randomized as individual units. The allocation sequence was created using a random number generator. Program sign-up, randomization, and group assignments were made by the study coordinator. The study was approved by the Institutional Review Board of the SwedishAmerican Health System on August 29, 2002.

Intervention

The intervention for this study was a live version of the Coronary Health Improvement Project (CHIP).[5] Participants met for four weeks—four times each week for 2 hours—where they received instruction. The curriculum included the following

topics: modern medicine and health myths, atherosclerosis, coronary risk factors, obesity, dietary fiber, dietary fat, diabetes, hypertension, cholesterol, exercise, osteoporosis, cancer, lifestyle and health, the Optimal Diet, behavioral change, and self-worth.

In conjunction with the CHIP lectures, participants received a textbook and workbooks that closely followed the discussion topics and contained assignments with learning objectives for every topic presented. These assignments were designed to help in the understanding and integration of the concepts and information presented. Dietitians and medical professionals spoke to the group weekly, introducing them to the latest nutritional and medical information related to the prevention of chronic diseases. Participants had access to scheduled shopping tours and cooking demonstrations given by a dietitian.

Participants were encouraged to follow pre-set dietary and exercise goals. The dietary goal involved adopting the more plant-food based diet that emphasizes "as-grown," unrefined food. Participants were encouraged to eat these foods: whole grains, legumes, vegetables, and fresh fruits. In addition, the diet was low in fat (less than 20% of energy), animal protein, sugar, salt, very low in cholesterol, and high in fiber. Concurrently, program participants were encouraged to progressively work toward walking or exercising for at least 30 minutes a day. Participants were given a pedometer and encouraged to keep an exercise log to record the miles walked each day. At the completion of the program, participants were encouraged to join the Rockford CHIP Alumni Organization for an annual cost of $25 for an individual or $35 for a couple.

The primary objectives of this therapeutic lifestyle change program were to improve participants': Cognitive understanding of the importance of healthy lifestyles; nutrition and physical activity behavior; risk factors associated with diabetes, hypertension, cardiovascular disease, and cancer.

Measures

Variables gathered included cognitive and behavioral measurements and physiological outcomes related to chronic disease. Demographic data was collected at baseline. Attendance at each of the classes was tracked and averaged. Participants attended 89 percent of the classes on average.

The intervention was designed to assist individuals in adopting healthy eating and physical activity behaviors. To assess dietary intake, the Block 98 full-length dietary questionnaire was used (Block 98.2, Block Dietary Data Systems, Berkeley, CA). The Block 98 questionnaire has been extensively studied and validated.[8] It is self-reported and optically scanned and scored. The variables measured by this survey include, but are not limited to, the following: daily nutrients from food, percent of calories, fiber from different sources, and food group servings per day.

To ascertain energy expenditure contributed by physical activity, a 7-day self-recorded pedometer log was maintained by each participant. Participants wore the Walk4Life Model 2000 Life Stepper pedometer (Plainfield, IL) on a belt at the right hip directly above the right knee cap each day for 7 days. Immediately prior to going to bed the pedometer counts for the day were recorded and the number reset. Strike counts from

pedometers are a valid and reliable method of monitoring and measuring free-living physical activity.[9]

The primary outcome variables for this study included several chronic disease risk factors. After a 12-hour fast, blood was drawn using a vacutainer (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) by phlebotomists from the SwedishAmerican Health System's outpatient laboratory. Samples were allowed to clot and centrifuged. Clinical analyses were completed at the SwedishAmerican Health System laboratory. Lipid analysis followed the lipid standards provided by the Centers for Disease Control and Prevention. Glucose, total cholesterol, HDL-cholesterol, and triglyceride concentrations are determined using Beckman-Coulter LX-20 instrumentation. Glucose was obtained using oxygen rate method employing Beckman oxygen electrode, cholesterol was obtained using timed endpoint enzymatic method using cholesterol oxidase, triglyceride used timed endpoint enzymatic method using glycerol kinase, and HDL was obtained using homogeneous timed endpoint method using polyanion detergent to separate HDL from non-HDL lipids. For participants with triglyceride values below 400, LDL values were calculated as follows: LDL = total cholesterol – HDL – (triglycerides/5).[10] High-sensitivity C-reactive protein (CRP) was determined using a microplate protocol based on a latex bead enhanced immunoturbidity assay.[11] Glucose was determined using a Kodak Ektachem. Trained program staff took blood pressure measures; after resting for five minutes, blood pressure was measured using the guidelines set forth by the American Heart Association. Weight and height were measured using standard medical weight and height scales recently calibrated by the

Biometrics Department of the SwedishAmerican Health System. Body mass index (BMI) was determined using the formula: weight (kg)/height (m^2).

Statistical Analyses

Cross-tabulations were used to perform bivariate analyses between selected variables, with statistical significance based on the chi-square test for independence. The t-test method was used for testing differences in means. Because multiple pair-wise tests were performed, an adjusted alpha should be referred to in order to minimize the overall probability of committing a type I error. The modified alpha based on the Bonferroni correction, 28 pair-wise tests, and alpha = 0.05 is 0.0001. This conservative alpha should be used when determining significance in Tables 2 and 3. Risk factor cut points (Tables 4 and 5) were previously established[12,13] and categorized accordingly. Results are based on the intent-to-treat method in which all participants were retained in the analyses. Where data were lost to follow-up, the participant's most recent available data used the last test carry forward method. The results did not differ significantly when those lost to follow-up were dropped from the analyses. These results are not reported in the paper. Analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA, 2003). Procedure statements used in SAS for assessing the data were PROC UNIVARIATE, PROC FREQ, PROC TTEST, and PROC GLM.

RESULTS

There were 318 participants who completed both baseline and 6-month evaluations. An additional 30 completed the baseline evaluation but not the 6-month

evaluation. Of these lost to follow-up, 21 were in the intervention group and 9 were in the control group (Figure 1).

Analyses were based on 348 participants. Ages ranged from 24 to 81 years, with little difference in the mean age between intervention and control groups (50.1 vs. 50.8, t-statistic = -.57, p = .5704. A description of participants in both intervention and control groups is presented according to selected demographic variables in Table 1. There were no statistically significant differences between groups for these variables. Within each variable the majority of participants were: female, white, married, had an annual family income of at least $60,000, and had at least some college education.

After six months, participants in the intervention group experienced significant improvements in each of the selected variables representing physical activity and nutrition (Table 2). Changes in the control group were generally not statistically significant or much smaller in magnitude. For each variable, the change observed in physical activity or nutrition was significantly greater for participants in the intervention group compared to the control group. The control group ate significantly more calories from fat and fewer servings of whole grains at six months.

After six months, participants in the intervention group showed significant improvements in cardiovascular risk factors for each of the variables considered except triglycerides and CRP, where the change scores were not significant (Table 3). Change scores through six months tended to be in the same direction for the control group, albeit only statistically significant for body fat, SBP, DBP, cholesterol, HDL, and LDL. For weight, body fat, DBP, resting heart rate, and HDL, changes were significantly greater

for participants in the intervention group compared to the control group. For some of the variables, differences in change scores were marginally insignificant.

Mean baseline, 6-month, and change scores are presented according to standard health risk cut points for the risk factor variables according to intervention (Table 4) and control status (Table 5). This analysis stratifies results according to risk status. Individuals with low risk would not be expected to experience large changes, but risk values considered to be high would be expected to change significantly. For the intervention group, the distributions favorably changed between baseline and 6 months for BMI, systolic blood pressure, and diastolic blood pressure. Corresponding significant change in the distribution between baseline and 6 months was not observed in the control group for BMI. Mean change scores within baseline risk categories tended to be significant for both intervention and control groups. However, favorable changes in risk behaviors were generally higher and more likely significant for those in the intervention group compared with the control group.

Whereas total cholesterol significantly increased between baseline and 6 months for participants in the control group, no significant difference was observed in the intervention group. For both intervention and control groups, cholesterol significantly increased among those with cholesterol in the normal range and significantly decreased for those with cholesterol in the high risk category. Cholesterol medication played a minimal role in the change observed in cholesterol. At baseline, there were 77 participants in the intervention group who reported being on blood pressure medication. At 6 months 60 (75%) indicated no change in their medication over the study period, 9 (11.2%) indicated a dosage increase, and 11 (13.8%) indicated a dosage decrease. There

was not a significant difference in the use of blood pressure medication from baseline to 6 months between the intervention and control groups ($\chi^2 = 1.14$, $p = 0.5636$).

DISCUSSION

Therapeutic lifestyle change can result in significant improvements in nutrition and physical activity behavior and reductions in many chronic disease risk factors. Six months after the intervention began, program participants continued to demonstrate dramatic improvements in nutrition and physical activity behavior. Increases in the number of servings of fruit and vegetables, whole grains, physical activity, and decreases in dietary sodium are likely responsible for the improvements in both systolic and diastolic blood pressure. Intervention group participants consumed 2.3 more servings of fruit and vegetables per day at six months as compared with baseline. In the PREMIER study,[14] participants who completed a behavior change program and adopted the DASH diet increased fruit and vegetable servings by 3.0 servings after six months. These PREMIER program participants decreased their percentage of calories from fat by 9.5% and lost an average of 5.8 kg of body weight. This compares to a percent fat reduction of 8.2% and a 4.5 kg weight loss for intervention participants in the present study.

The intervention group had a 44% reduction in the number of participants who were at least diastolic prehypertensive at follow-up and a 20% reduction in the prevalence of systolic pressures that were at least prehypertensive at baseline. The average reductions in blood pressure were greater than those reported in the DASH feeding study,[15] and comparable to the results of the PREMIER Clinical feeding trial.[14]

Despite sharp improvements in blood lipids at six weeks, these changes disappeared at six months.[7] Other therapeutic lifestyle trials that lasted longer than three

months and included lipid outcomes reported similar findings.[16-19] Dietary cholesterol was reduced by 122 mg/day (56% reduction) for program participants, and dietary saturated fat was cut by half. Despite these favorable changes in dietary cholesterol precursors, a return to prior lipid levels suggests that there is a significant increase in endogenous cholesterol, most of which appears to be LDL cholesterol.[20] Without more accurate measures of endogenous cholesterol biosynthesis it is impossible to determine the exact cause of the cholesterol increase.[21]

Pedometer data shows that the program participants increased physical activity by 30%. The average number of steps for the intervention group after six months did not meet the recommended 10,000 steps per day.[22] However, for this predominately middle-aged, predominately obese population, an increase in physical activity of this magnitude likely contributed to risk factor reductions. When combined with diet changes, improvement in physical activity is the likely explanation for decreases in BMI (5%), weight (5%), and percent body fat (6%). Improved physical activity was also associated with a significant decrease in resting heart rate, a correlated measure of cardiorespiratory fitness thought to be caused by increased heart size, blood volume, stroke volume, and cardiac output.[23]

Poor nutrition and sedentary living are associated with a constellation of risk factors, some identified in the metabolic syndrome, and all linked to common chronic diseases.[24] Improvements in nutrition and physical activity are associated with significant improvements in diabetes risk as whole body glucose tolerance improves, insulin sensitivity increases, and the amount of glucose transporter (GLUT4) increases.[25] The number of diabetics (glucose $\geq$ 126) in the intervention group was reduced by 19%,

demonstrating that this therapeutic lifestyle change program improve insulin sensitivity. Similar results were reported by other lifestyle trials reporting glucose findings.[16-19]

These improvements in behavior and risk are not unexpected. In the intervention, participants attended highly interactive lectures structured around the health belief and transtheoretical models. Video clips, testimonials, role playing, short presentations from physicians, social support strategies, food selection and planning activities, and other behavior change- driven pedagogical activities all helped to encourage participants to enthusiastically evaluate personal behaviors and commit to make changes. To prevent relapse and help participants maintain their new behaviors, the CHIP program graduates were invited to participate in the CHIP alumni program. Alumni received a monthly newsletter which contains news of health-promoting community events such as healthy dinners, walking groups, and support group meetings. The alumni are encouraged to attend special lectures on healthy living and ways to avoid relapse.

The participants were mostly white and sufficiently self-motivated to volunteer to participate in the intervention. On average, participants were slightly more educated than the community average. Participants had lifestyles that permitted them to attend most, if not all, of the classes. This is evident in the high rate of attendance to this time intensive program. These delimitations do threaten the generalizability of these findings and makes application of the intervention to other populations problematic. Since the participants were self-selected, the results from this intervention may represent a “best-case” scenario.

Despite the apparent effect of this intervention, there are some shortcomings associated with the study design. Both the physical activity and nutrition data were self-

reported. For some variables, the control group also experienced significant improvement. Significant decreases were observed in this group in percentage of calories from fat and dietary fat grams, sodium grams, and total calories as well as small increases in total steps. In addition, the control group experienced similar improvement in blood pressure compared with the intervention group. Because the control group was asked to wait six months before beginning the program, it is possible that members of the control group anticipated program participation and began to make behavior changes on their own. Furthermore, there are over 27 restaurants in the Rockford metropolitan area that offer healthy, "CHIP" menu items which could have contributed to improvements in the control group.

The results of this study indicate that a community-based intervention, which uses various behavior modification tools, such as live lectures, workbooks, and professional advice, can result in reduced risk factors for cardiovascular disease after six months in a middle-aged population. Further research is needed to examine the effects of the program on other populations.

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Figure 1. Flow diagram of participant process

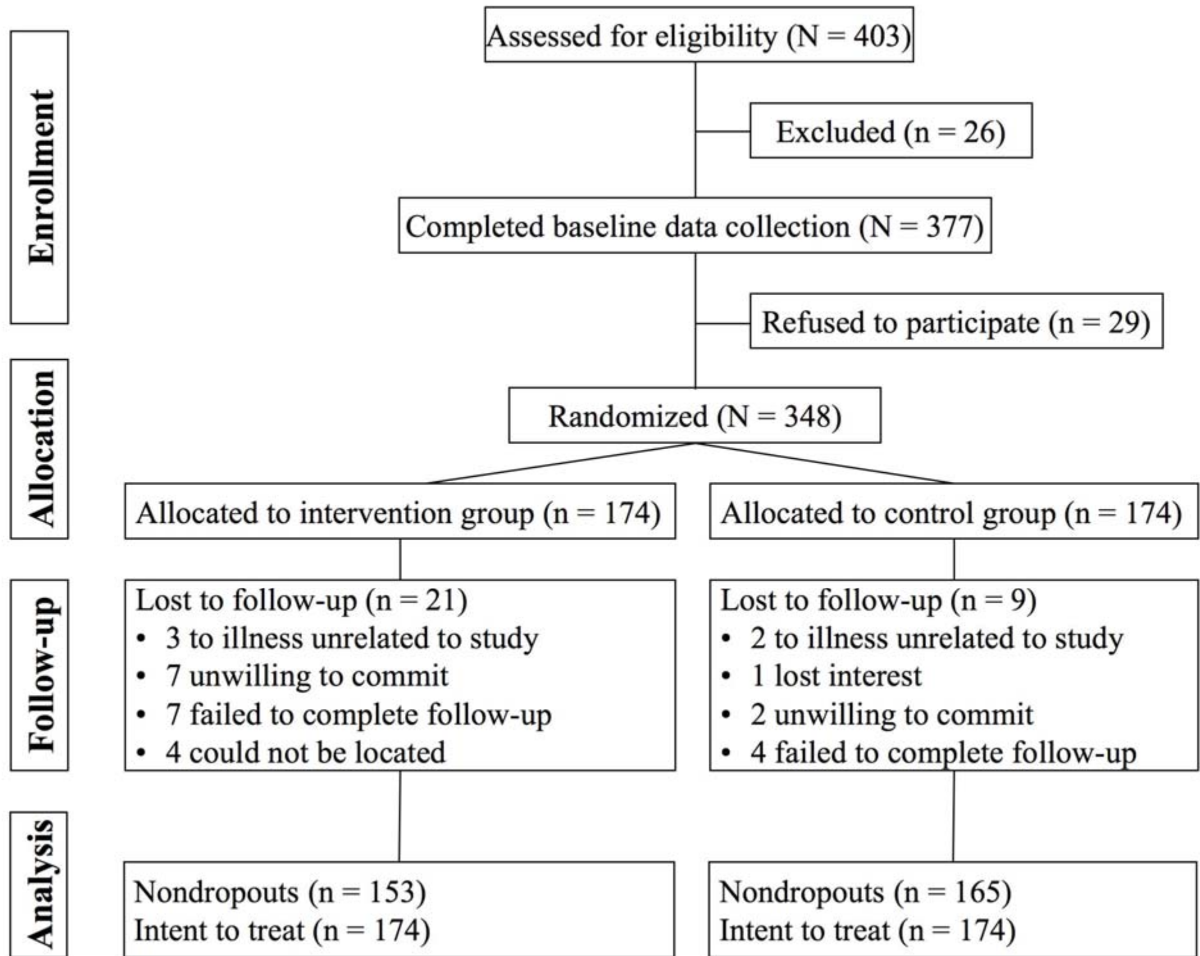

Table 1. Mean and Frequency Distributions for Selected Demographic Variables According to Intervention Status

	Intervention		Control		χ^2
	No.	%	No.	%	P Value
Gender					
Male	47	27.0	51	29.3	0.227
Female	127	73.0	123	70.7	0.634
Race					
White alone	167	96.0	160	93.0	
Black alone	4	2.3	10	5.8	2.910
Other	3	1.7	2	1.2	0.233
Marital Status					
Never Married	12	6.9	20	11.6	
Married	138	79.8	127	73.4	
Divorced	16	9.2	16	9.2	2.986
Widowed	7	4.1	10	5.8	0.394
Annual Family Income					
$0–$20,000	14	8.2	12	7.1	
$20,001–$40,000	34	20.0	28	16.5	
$40,001–$60,000	37	21.8	41	24.1	1.032
$60,000+	85	50.0	89	52.3	0.7936
Education					
< High School	4	2.3	7	4.0	
High School	37	21.5	46	26.6	
Some College	58	33.7	39	22.5	
College Degree	39	22.7	38	22.0	6.578
Post College Degree	34	19.8	43	24.9	0.160

Table 2. Mean (Standard Deviation) of Physical Activity and Nutrition Variables at Baseline and 6 Months

	Intervention (n=174)				Control Group (n=174)					
	Baseline	6 Months	Mean Change	P value	Baseline	6 Months	Mean Change	P value	Corrected Difference* (95% CI)	P Value at 6 Months
Total steps per week	40579 (22631)	52951 (24240)	12372	<.0001	43869 (23466)	49530 (22544)	5661	<.0001	6711 (3026, 10396)	.0002
Total energy,	2092 (1030)	1534 (691)	-558	<.0001	1919 (805)	1773 (777)	-146	.0010	-412 (-556, -271)	<.0001
Calories from fat, %	36.7 (6.9)	28. (7.0)	-8.2	<.0001	34.6 (7.4)	35.6 (8.3)	1	.0569	-9.2 (-10.6, -7.6)	<.0001
Calories from protein, %	15.2 (2.8)	14.4 (2.2)	-0.8	.0003	14.7 (2.5)	15.4 (3.2)	.7	.0097	-1.5 (-2.1, -0.8)	<.0001
Carbohydrates, %	48.7 (8.0)	59.2 (8.5)	10.5	<.0001	50.8 (8.2)	49.4 (9.6)	-1.4	.0168	11.9 (10.1, 13.6)	<.0001
Fruit & veg. fiber g/day	7.6 (4.3)	11.6 (5.6)	4	<.0001	8.3 (5.0)	8.0 (4.5)	-0.3	.1910	4.3 (3.3, 5.3)	<.0001
Vegetable servings/day	3.3 (2.1)	4.7 (2.6)	1.4	<.0001	3.4 (2.2)	3.5 (2.1)	0.1	.6635	1.3 (0.9, 1.9)	<.0001
Fruit servings/day	1.3 (1.0)	2.2 (1.2)	0.9	<.0001	1.6 (1.1)	1.6 (1.1)	0	.6948	0.9 (0.6, 1.1)	<.0001
Whole grain servings/day	5.4 (2.9)	6.1 (3.2)	0.7	.0022	5.0 (2.4)	4.5 (2.3)	-0.5	.0007	1.2 (0.7, 1.7)	<.0001
Meat servings/day	2.1 (1.4)	1.3 (1.0)	-0.8	<.0001	1.9 (1.2)	1.9 (1.1)	0	.9884	-0.8 (-1.0, -0.5)	<.0001
Dietary fat g/day	88.6 (55.3)	50.6 (33.5)	-38	<.0001	76.8 (42.9)	71.9 (40.3)	-4.9	.0298	-33.1 (-40.6, -25.7)	<.0001
Dietary cholesterol, mg	216 (140)	94 (90)	-122	<.0001	182 (112)	192 (140)	10	.2170	-132 (-153, -108)	<.0001
Polyunsaturated fat, g	21.2 (14.0)	13.6 (8.3)	-7.6	<.0001	19.3 (12.0)	17.7 (10.4)	-1.6	.0123	-6.0 (-8.1, -4.1)	<.0001
Monounsaturated fat, g	34.3 (21.6)	18.8 (13.1)	-15.5	<.0001	29.7 (17.2)	27.9 (16.3)	-1.8	.0480	-13.7 (-16.6, -10.6)	<.0001
Saturated fat, g	26.3 (17.3)	13.3 (10.5)	-13	<.0001	21.8 (12.1)	20.5 (12.0)	-1.4	.0311	-11.6 (-13.9, -9.3)	<.0001
Sodium, mg	2941 (1530)	2332 (1216)	-609	<.0001	2712 (1233)	2486 (1135)	-226	.0008	-383 (-590, -176)	.0003

Abbreviations: CI, confidence interval.
*Difference in change through 6 months between intervention and control groups.

Table 3. Mean (Standard Deviation) of Cardiovascular Risk Factors at Baseline and 6 Months

	Intervention (n=174)				Control Group (n=174)					
	Baseline	6 Months	Mean Change	P value	Baseline	6 Months	Mean Change	P value	Corrected Difference (95% CI) *	P Value at 6 Months
Body Mass Index	33.3 (8.0)	31.7 (8.1)	-1.6	<.0001	31.4 (9.0)	31.1 (9.2)	-0.3	.0031	-1.3 (-1.65, -0.96)	<.0001
Weight, kg	93.3 (24.1)	88.8 (24.0)	-4.5	<.0001	87.7 (25.9)	87.1 (26.0)	-0.6	.1019	-3.9 (-5.0, -2.8)	<.0001
Body fat, %	40.6 (8.8)	38.2 (9.6)	-2.4	<.0001	37.9 (10.3)	37.1 (10.5)	-0.8	.0016	-1.6 (-2.3, -0.9)	<.0001
SBP, mm Hg	129 (16)	124 (18)	-5	<.0001	128 (17)	124 (18)	-4	<.0001	-1 (-4, 2)	.5068
DBP, mm Hg	78.3 (9.2)	72.8 (9.7)	-5.5	<.0001	76.7 (9.6)	72.9 (9.7)	-3.8	<.0001	-1.7 (-3.5, -0.0)	.0427
Resting heart rate, bts/min	73.1 (10.2)	69.6 (10.6)	-3.5	<.0001	72.1 (10.6)	70.8 (10.3)	-1.3	.1299	-2.2 (-4.4, -0.1)	.0291
Glucose, mg/dL	103 (23)	100 (20)	-3	.0058	100 (19)	99 (22)	-1	.7731	-2 (-6, 0.4)	.0861
Cholesterol, mg/dL	193 (33)	199 (34)	6	.0045	190 (39)	201 (39)	11	<.0001	-5 (-11, 1)	.0753
HDL, mg/dL	45.0 (12.2)	46.4 (11.8)	1.4	.0076	45.0 10.4)	47.8 (10.4)	1.8	<.0001	-1.4 (-2.9, -0.0)	.0454
LDL, mg/dL	122 (29)	127 (29)	5	.0052	121 (33)	130 34)	9	<.0001	-4 (-9, 2)	.1604
Triglycerides, mg/dL	133 (102)	128 (78)	-5	.3666	115 (86)	117 (69)	2	.7568	-7 (-22, 9)	.4047
CRP, mg/dL†	283.0 (2.6, 1320)	217.5 (0.2, 1419)	-66.5	.0631	228.6 (13.5, 1356.8)	226.6 (0.4, 1354.5)	-2.0	.1662	-68.5	.4002

Abbreviations: CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein, LDL, low-density lipoprotein; CRP, C-reactive protein.
*Difference in change through 6 months between intervention and control groups.
†CRP mean change scores violated the assumption of normality. Thus, median and range scores are reported. The Wilcoxon Signed-Ranks Tests was used to test for differences in medians within groups and the Wilcoxon Rank-Sum Test was used to test for differences in change between groups.

Table 4. Health Risk Prevalence and Change Scores through Six Months for the Intervention Group

	Baseline (n = 174)		Six Months (n = 174)						
	No.	%	No.	%	χ^2 (trend) P Value†	Baseline Mean	Follow-up Mean‡	Mean Change	T Statistic P Value
BMI (Kg/m²)									
Underweight (< 18.5)	0	0.00	1	0.57	0.0447	---	---	---	---
Normal (18.5-24.9)	24	13.79	36	20.69		22.74	21.90	-0.84	0.0257
Overweight (25.0-29.9)	45	25.86	47	27.01		27.70	26.14	-1.56	< 0.0001
Obese (≥ 30.0)	105	60.34	90	51.72		38.16	36.35	-1.81	< 0.0001
Systolic BP (mmHg)									
Normal (<120)	52	29.89	76	43.68	0.0250	111.12	109.51	-1.61	0.2792
Pre hyperten. (120-139)	79	45.40	64	36.78		129.22	123.88	-5.34	0.0002
High (140-159)	35	20.11	28	16.09		147.56	137.60	-9.96	< 0.0001
Dangerous (≥160)	8	4.60	6	3.45		167.50	158.62	-8.88	0.0464
Diastolic BP (mmHg)									
Normal (<80)	97	55.75	131	75.29	< 0.0001	71.90	68.09	-3.81	< 0.0001
Pre hypertensive (80-89)	55	31.61	35	20.11		83.31	78.58	-4.73	< 0.0001
High (90-99)	20	11.49	7	4.02		93.25	76.80	-16.45	< 0.0001
Dangerous (≥100)	2	1.15	1	0.57		---	---	---	---
Total CHL (mg/dL)									
Normal (<200)	105	60.34	94	54.02	0.2019	171.93	183.69	11.76	< 0.0001
Borderline (200-239)	54	31.03	60	34.48		215.92	218.92	3.00	0.3477
High risk (≥240)	15	8.62	20	11.49		257.67	234.93	-22.74	0.0008
LDL (mg/dL)									
Optimal (<100)	42	24.14	30	17.24	0.0786	85.02	103.07	18.05	< 0.0001
Above optimal (100-129)	70	40.23	69	39.66		115.73	121.94	6.21	0.0161
Borderline (130-159)	45	25.86	53	30.46		143.27	145.18	1.91	0.5394
High (160-189)	14	8.05	17	9.77		172.64	151.43	-21.21	0.0002
Very high (≥190)	3	1.72	5	2.87		---	---	---	---
HDL (mg/dL)									
High (≥60)	19	10.92	20	11.49	0.0640	69.16	68.21	-0.95	0.5224
Normal (40-59)	84	48.28	104	59.77		48.52	48.78	0.26	0.8118
Low (<40)	71	40.80	50	28.74		34.33	37.87	3.54	< 0.0001
Triglycerides (mg/dL)									
Normal (<150)	124	71.26	129	74.14	0.5650	88.68	100.05	11.37	0.0175
Borderline (150-199)	25	14.37	22	12.64		171.52	152.58	-18.94	0.1003
High (200-499)	23	13.22	22	12.64		283.22	238.30	-44.92	< 0.0001
Very high (≥500)	2	1.15	1	0.57		---	---	---	---
Glucose (mg/dL)									
Normal (<110)	136	78.16	141	81.03	0.4644	93.67	92.77	-0.90	0.4351
IFG (110-125)	17	9.77	16	9.20		114.41	108.56	-5.85	0.0793
Diabetes (≥126)	21	12.07	17	9.77		152.00	137.90	-14.10	< 0.0001

†Mantel-Haenszel chi-square test.
‡Follow-up means are from the same individuals in each baseline risk category. IFG=impaired fasting glucose, BP=blood pressure, CHL=cholesterol, HDL=high density lipoprotein, LDL=low density lipoprotein

Table 5. Health Risk Prevalence and Change Scores through Six Months for the Control Group

	Baseline (n = 174)		Six Months (n = 174)						
	No.	%	No.	%	χ^2 (trend) P Value†	Baseline Mean	Follow-up Mean‡	Mean Change	T Statistic P Value
BMI (Kg/m^2)									
Underweight (< 18.5)	0	0.00	1	0.57	0.8435	---	---	---	---
Normal (18.5-24.9)	43	24.71	44	25.29		23.15	23.04	-0.11	0.6015
Overweight (25.0-29.9)	56	32.18	54	31.03		27.42	27.10	-0.32	0.1225
Obese (≥ 30.0)	75	43.10	75	43.10		39.11	38.62	-0.49	0.0051
Systolic BP (mmHg)									
Normal (<120)	60	34.48	81	46.55	0.0303	111.07	109.67	-1.40	0.3590
Pre hypertensive (120-139)	69	39.66	64	36.78		128.72	123.52	-5.20	0.0004
High (140-159)	40	22.99	21	12.07		149.51	141.64	-7.87	< 0.0001
Dangerous (≥160)	5	2.87	8	4.60		165.40	157.00	-8.40	0.1130
Diastolic BP (mmHg)									
Normal (<80)	102	58.62	127	72.99	0.0023	70.55	68.08	-2.47	0.0014
Pre hyperten. (80-89)	52	29.89	40	22.99		82.21	77.83	-4.38	< 0.0001
High (90-99)	18	10.34	5	2.87		93.33	84.61	-8.72	< 0.0001
Dangerous (≥100)	2	1.15	2	1.15		---	---	---	---
Total CHL (mg/dL)									
Normal (<200)	107	61.49	86	49.43	0.0095	164.20	183.74	19.54	<0.0001
Borderline (200-239)	52	29.89	60	34.48		219.77	227.90	8.13	0.0427
High risk (≥240)	15	8.62	28	16.09		260.47	236.67	-23.8	0.0020
LDL (mg/dL)									
Optimal (<100)	48	27.59	34	19.54	0.0122	81.43	100.38	18.95	< 0.0001
Above optimal (100-129)	58	33.33	52	29.89		113.48	130.12	16.64	< 0.0001
Borderline (130-159)	46	26.44	50	28.74		142.67	148.11	5.44	0.1254
High (160-189)	17	9.77	32	18.39		170.35	169.06	-1.29	0.7782
Very high (≥190)	5	2.87	6	3.45		---	---	---	---
HDL (mg/dL)									
High (≥60)	16	9.20	24	13.79	0.0226	65.06	62.75	-2.31	0.1544
Normal (40-59)	99	56.90	109	62.64		48.05	50.08	2.03	0.0013
Low (<40)	59	35.91	41	23.56		33.87	39.84	5.97	< 0.0001
Triglycerides (mg/dL)									
Normal (<150)	138	79.31	131	75.29	0.3952	84.02	99.76	15.74	0.0015
Borderline (150-199)	18	10.34	21	12.07		171.50	154.72	-16.78	0.2147
High (200-499)	17	9.77	21	12.07		259.12	208.12	-51.00	0.0003
Very high (≥500)	1	0.57	1	0.57		---	---	---	---
Glucose (mg/dL)									
Normal (<110)	150	86.21	152	87.36	0.7900	94.64	94.41	-0.23	0.7660
IFG (110-125)	17	9.77	13	7.47		112.94	115.56	2.62	0.5003
Diabetes (≥126)	7	4.02	9	5.17		175.57	168.33	-7.24	0.2479

†Mantel-Haenszel chi-square test.
‡Follow-up means are from the same individuals in each baseline risk category. IFG=impaired fasting glucose, BP=blood pressure, CHL=cholesterol, HDL=high density lipoprotein, LDL=low density lipoprotein

THE EFFECTS OF A WORKSITE CHRONIC DISEASE PREVENTION PROGRAM

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ABSTRACT

BACKGROUND

This study determined the behavioral and clinical impact of a worksite chronic disease prevention program.

DESIGN

Randomized clinical trial.

SETTING/PARTICIPANTS

Participants included 145 employees and spouses of the SwedishAmerican Health System in Rockford, Illinois.

INTERVENTION

The intervention group attended a 40-hour lecture educational course delivered over a four-week period.

MAIN OUTCOME MEASURES

Nutrition and physical activity behavior and several chronic disease risk factors assessed at baseline, 6 weeks, and 6 months.

RESULTS

Cognitive understanding of the requirements for a healthy lifestyle increased at the end of the program. Program participants significantly improved cognitive understanding of good nutrition and physical activity and had significantly better nutrition and physical activity behavior at both 6 weeks and 6 months. After six months, daily fruit and vegetable servings had increased an average of 2.1 servings. Participants had significantly lower body fat, blood pressure, and cholesterol.

CONCLUSIONS

This worksite chronic disease prevention program can significantly increase health knowledge, can improve nutrition and physical activity, and can improve many employee health risks in the short term.

INTRODUCTION

In 2003, health care costs for companies across the United States increased an average of 14%, the largest single-year increase since 1990.[1] In that same year, the premiums for employer-sponsored health plans rose to $3,383 for single coverage and $9,068 for family coverage.[1] As companies continue to bear these costs, many initiate employee health promotion programs in an attempt to help employees prevent chronic disease and hopefully reduce medical care expenses. This is especially important for worksites that are self-insured. Approximately 90% of all workplaces in the United States with 50 or more employees have some form of health promotion program.[2]

The SwedishAmerican Health System (SAHS) is the largest medical care provider in Rockford, Illinois, and employs 2,744 medical personnel and staff. Like many other worksites, SAHS offers its employees medical care insurance but is also the provider of that medical care and is not immune to the increased medical costs experienced by other employers. Because of the increased medical care cost burden and its desire to improve the health if its employees, SAHS initiated an employee wellness program. One component of the wellness program was the adoption of the Coronary Health Improvement Project (CHIP). The CHIP program is a 40-hour, live-lecture educational course that highlights the importance of making better lifestyle choices for preventing chronic diseases. It was created with the goal of reducing chronic diseases and improving the overall health of the public.[3] To determine if SAHS employees who participated in the program could improve their cognitive understanding of good health, healthy behaviors, and chronic disease risk factors through 6 weeks and 6 months, a large randomized clinical trial was initiated.

METHODS

Subject Recruitment and Design

Recruitment efforts included "lunch and learn" presentations, intranet communications, posting flyers, word of mouth, and newsletter announcements to all SAHS employees. To be included in the study, each participant had to be willing to participate in the program starting in one month or in seven months. To encourage participation, SAHS shared the cost of program participation. SAHS paid $295 of the initial $395 program cost with employees responsible to pay the $100 difference. Upon successful completion of the program, SAHS refunded the $100 employee portion, so there was no cost. Employees were further incentivised for participation by being paid an additional $100; and, if they were diabetic, they were paid another $100. Because spouses of employees are also covered by the SAHS health plan, they too were encouraged to participate. Insured spouses paid $100 and the health plan paid the balance after successful completion.

Eligible and interested participants provided informed consent. Participants were encouraged to participate with a spouse or significant other. If an employee decided to participate with a partner, the employee and the partner were randomized as a paired unit. All other participants were randomized as individuals. The allocation sequence was created using a random number generator. Program sign-up, randomization, and group assignments were made by the study coordinator. The study was approved by the Institutional Review Board of the SwedishAmerican Health System on August 29, 2002.

Intervention

The intervention for this study was a live version of the Coronary Health Improvement Project (CHIP).[3] Participants met for four weeks—four times each week for 2 hours each session—where they received instruction. Meetings were held off-site at a local college. The curriculum included the following topics: modern medicine and health myths, atherosclerosis, coronary risk factors, obesity, dietary fiber, dietary fat, diabetes, hypertension, cholesterol, exercise, osteoporosis, cancer, lifestyle and health, the optimal diet, behavioral change, and self-worth.

In conjunction with the CHIP lectures, participants received a textbook and workbooks that closely followed the discussion topics and contained assignments with learning objectives for every topic presented. These assignments were designed to help in the understanding and integration of the concepts and information presented. Dietitians and medical professionals spoke to the group weekly, introducing them to the latest nutritional and medical information related to the prevention of chronic diseases. In addition, participants had access to scheduled shopping tours and cooking demonstrations given by a dietitian. Finally, the lecturer and program staff presided at each of the educational sessions and were available to answer questions regarding the presentations, workbook assignments, and the program.

Participants were encouraged to follow pre-set dietary and exercise goals. The dietary goal involved adopting a more plant-food based diet that emphasizes "as-grown," unrefined food. Participants were encouraged to increase their consumption of whole grains, legumes, vegetables, and fresh fruits. The recommended diet was low in fat (less than 20% of energy), animal protein, sugar, and salt, very low in cholesterol, and high in fiber. Concurrently, program participants were encouraged to progressively work toward

walking or other exercises at least 30 minutes a day. Participants were given a pedometer and encouraged to keep an exercise log to record the miles walked each day. At the completion of the program, participants were encouraged to join the Rockford CHIP Alumni Organization for after-program support.

The primary objectives of this program are to improve participants' cognitive understanding of the importance of healthy lifestyles, nutrition and physical activity behavior, risk factors associated with diabetes, hypertension, cardiovascular disease, and cancer.

Measures

Variables included cognitive and behavioral measurements and physiological outcomes related to chronic disease. Demographic data was collected at baseline. Attendance at each of the classes was tracked and averaged. Participants attended 87% of the classes on average. A first step in behavior change may involve increasing awareness of proper health behaviors and knowledge. Specific knowledge regarding health risks, proper eating and physical activity behaviors, and an understanding of the benefits of a healthy lifestyle was assessed with a multiple-choice health knowledge test. The test has previously demonstrated validity and reliability.[3] Information about each of the 30 questions is contained in the workbook, handout materials, and in a CHIP video lending library, so that participants who missed a lecture could obtain the information. The percent of correct responses was recorded at baseline and after 6 weeks.

To assess dietary intake, the Block 98 full-length dietary questionnaire was used (Block 98.2, Block Dietary Data Systems, Berkeley, CA). This questionnaire has been

extensively studied and validated.[4-6] It is self-reported and optically scanned and scored. The variables measured by this survey include, but are not limited to, the following: daily nutrients from food, percent of calories, fiber from different sources, and food group servings per day.

To ascertain energy expenditure contributed by physical activity, a 7-day self-recorded pedometer log was maintained by each participant. Participants wore the Walk4Life Model 2000 Life Stepper pedometer (Plainfield, IL) on a belt at the right hip directly above the right knee cap each day for 7 days. Immediately prior to going to bed, the pedometer counts for the day were recorded and the number reset. Strike counts from pedometers are a valid and reliable method of monitoring and measuring free-living physical activity.[7-9]

The primary outcome variables included several chronic disease risk factors. After a 12-hour fast, blood was drawn using a vacutainer (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) by phlebotomists from the SwedishAmerican Health System's outpatient laboratory. Samples were allowed to clot and centrifuged. Clinical analyses were completed at the SwedishAmerican Health System laboratory. Lipid analysis followed the lipid standards provided by the Centers for Disease Control and Prevention. Glucose, total cholesterol, HDL-cholesterol, and triglyceride concentrations were determined using Beckman-Coulter LX-20 instrumentation. Glucose was obtained using oxygen rate method employing Beckman oxygen electrode, cholesterol was obtained using timed endpoint enzymatic method using cholesterol oxidase, triglyceride used timed endpoint enzymatic method using glycerol kinase, and HDL was obtained using homogeneous timed endpoint method using polyanion detergent to separate HDL from

non-HDL lipids. For participants with triglyceride values below 400 mg/dL, LDL values were calculated as follows: LDL = total cholesterol – HDL – (triglycerides/5).[10-12] High-sensitivity C-reactive protein (CRP) was determined using a microplate protocol based on a latex bead enhanced immunoturbidity assay.[13,14] Trained program staff took blood pressure measures; after resting for five minutes, blood pressure was measured using the guidelines set forth by the American Heart Association.[15] Weight and height were measured using standard medical weight and height scales recently calibrated by the Biometrics Department of the SwedishAmerican Health System. Body mass index (BMI) was determined using the formula: weight (kg)/height (m^2).

STATISTICAL ANALYSES

Cross-tabulations were used to perform bivariate analyses between selected demographic variables, with statistical significance based on the chi-square test for independence (χ^2).[16] Differences in independent means were evaluated using the t-test.[17] Wilks' lambda was used to evaluate differences in means across time between intervention and control groups.[18] Analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA, 2003). Procedure statements used in SAS for assessing the data were PROC UNIVARIATE, PROC FREQ, PROC TTEST, and PROC GLM. Statistical significance was based on the 0.05 level.

RESULTS

Of the 145 randomized participants, 8 were lost to follow-up (Figure 1). Drop out rates between groups were similar, and the analysis was based on intent-to-treat. Mean

ages did not significantly differ between intervention (M = 46.1, SD = 10.8) and control (M = 45.9, SD = 9.3) participants (t-statistic p = 0.9091). Descriptions of participants in the intervention and control groups are presented according to gender, race, marital status, income, and education in Table 1. There were no statistically significant differences between participants in the intervention and control groups for these variables. The majority of participants were: female, white, married, had an annual family income above $60,000, and had at least some college education.

The intervention group scored 64% on the cognitive baseline test. At the completion of the program the same participants averaged 95%. Baseline means and mean change scores for selected physical activity and nutrition variables are presented according to intervention status in Table 2. Mean change scores within groups tended to be significantly greater for the intervention group compared with the control group at both 6 weeks and 6 months. However, for a few variables, significant differences in change scores were observed at 6 weeks but not at 6 months (total steps and whole grains). For other variables, significant differences in mean change scores were not observed at 6 weeks but they were seen at 6 months (i.e., total energy, meat servings, polyunsaturated fat, and sodium). The effect of time on mean scores is significantly different between the intervention and control groups for each of the variables.

Baseline means and mean change scores for selected cardiovascular risk factor variables are presented according to intervention status in Table 3. Significantly more pronounced decreases in mean scores were observed in the intervention group compared with the control group at both 6 weeks and 6 months for BMI, weight, body fat, cholesterol, and HDL. Between-group mean change scores were significant at 6 weeks

but not 6 months for resting heart rate and LDL. None of the variables had significant between-group mean scores at 6 months that had insignificant between-group mean scores at 6 weeks. The effect of time on mean scores is significantly different between the intervention and control groups for BMI, weight, body fat, resting heart rate, cholesterol, HDL, and LDL.

Many of the improvements in health behavior showed large 6-week improvements that diminished somewhat after 6 months, but still remained significantly better than baseline. For example, the average number of steps as measured by pedometer increased and peaked at 6-weeks. This was a 25% increase in steps from baseline. By 6 months, this increase had dropped to a 16% overall increase from baseline. Step counts for the control group were essentially unchanged during the same period. Similarly, intervention participants reported vegetable serving consumption increased from 3.2 servings per day at baseline to 4.8 servings per day at 6 weeks, a 50% increase. By 6 months, vegetable servings consumed per day still averaged 4.7 servings with only a slight decline from the 6 week peak. Vegetable consumption in the control group was unchanged.

DISCUSSION

Employees who participated in this intensive lifestyle change program improved their health knowledge, adopted and maintained healthy eating and physical activity behaviors, and experienced favorable improvements in many chronic disease risk factors. SAHS was able to improve the health of many of its employees by encouraging them to participate in this lifestyle change program. Participants in the control group were

allowed to participate in the CHIP program after completing the 6-month follow up period.

At 6 weeks, many participants may have reached the maximum amount of improvement for many behaviors including steps, fruit and vegetable fiber, fruit servings, and whole grain servings. Healthy behavior maintenance or continued improvement was seen in percent of calories from fat, meat servings, dietary fat grams, dietary cholesterol, saturated fat, and sodium. Campbell et al. also reported significant increases in fruit and vegetable consumption at 6 months, but no differences after 18 months.[19] Reductions in the percent of dietary calories from fat and increases in fruits and vegetable servings reported were three and two times greater, respectively, than similar measures improvements reported by Stevens et al.[20] who used an equally short follow-up time and counseling sessions to improve nutrition among working adults. These greater improvements in behavior from SAHS employees are probably due to the more intense nature of the CHIP program.

Worksite nutrition trials such as 5 a Day for Better Health,[21] Working Healthy Project,[22] Next Step Trial,[23] Working Well Trial,[24] Health Works for Women,[19] and others[25-27] all had follow up periods of at least 12 months, and many lasted for 2 years. Most of these trials were able to show significant short-term improvement in nutrition, but after 2 years, healthy nutrition behaviors had attenuated to the point that there was either no significant change over baseline or only modest improvements. It would appear that worksite nutrition interventions can cause short-term behavior change, but as with other healthy human behaviors that are new, long-term compliance is difficult. Even

though the SAHS employees were able to demonstrate dramatic changes after six months, long-term behavior compliance is yet to be determined.

SAHS employees were able to increase pedometer measured baseline physical activity by 25% at 6 weeks and by 16% at 6 months. The average number of steps for the intervention group after six months did not meet the recommended 10,000 steps per day.[28] However, when combined with dietary changes, improvements in physical activity are the likely explanation for 6 month decreases in weight (5%) and percent body fat (6%). Improved physical activity was also associated with significant decreases in resting heart rate, a correlated measure of cardiorespiratory fitness thought to be caused by increased heart size, blood volume, stroke volume, and cardiac output.[29,30] These increases in physical activity are in agreement with what Proper et al.[31] found in their review of 26 worksite physical activity interventions.

Total cholesterol levels were dramatically lower at 6 weeks, however, despite significant 6 month reductions in dietary saturated fat and cholesterol, blood cholesterol returned to baseline values. A return to baseline lipid levels in the presence of reduced dietary cholesterol precursors suggests that program participants must be experiencing a significant increase in endogenous cholesterol, most of which appears to be LDL cholesterol.[32] Other lifestyle trials that lasted longer than three months have reported similar findings.[33-36]

SAHS employees who completed the program were able to demonstrate significant reductions in blood pressure and body fat. From baseline to 6 months there was a 31% increase in the number of participants who were classified as systolic normotensive (systolic blood pressure $\leq$120 mmHg) and a 46% increase in the number of

participants who were classified as diastolic normotensive (diastolic blood pressure $\leq$80 mmHg). Corresponding percentages for the control group are 25% and 9%, respectively. Theses findings are similar to those reported in the PREMIER Clinical feeding trial.[37]

At 6 months the number of obese program participants decreased from 32 at baseline to 27. In the control group, 2 of the 33 members of the control group who were obese at baseline no longer had a BMI $\geq$ 30 kg/m^2 at 6 months. Reductions in excessive weight continued through the 6 month period. Participants averaged 1.5 pounds of weight loss per month, which is ideal for individuals who are trying to maintain long-term healthy weight. The American College of Sports Medicine recommends that weight loss not exceed 1–2 pounds per month.[38]

The participants in this study were mostly white and sufficiently self-motivated to volunteer to participate in the intervention. This delimitation threatens the generalizability of these findings. Both the physical activity and nutrition data were self-reported and the follow-up period of the study was short. SAHS employees who were members of the control group also experienced some improvement in nutrition and physical activity behaviors and blood pressure. Because the control group was asked to wait six months before beginning the program, it is possible that members of the control group anticipated program participation and began to make behavior changes on their own.

The results of this study indicate that a worksite lifestyle change intervention can improve nutrition and physical activity behavior and can reduce many chronic disease risk factors. These findings add to the growing body of evidence that recognizes worksite efforts to improve nutrition and physical activity behavior and reduce many

chronic disease risk factors. Further research is needed to determine how long these beneficial changes will last.

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Figure 1. Flow diagram of participant progress

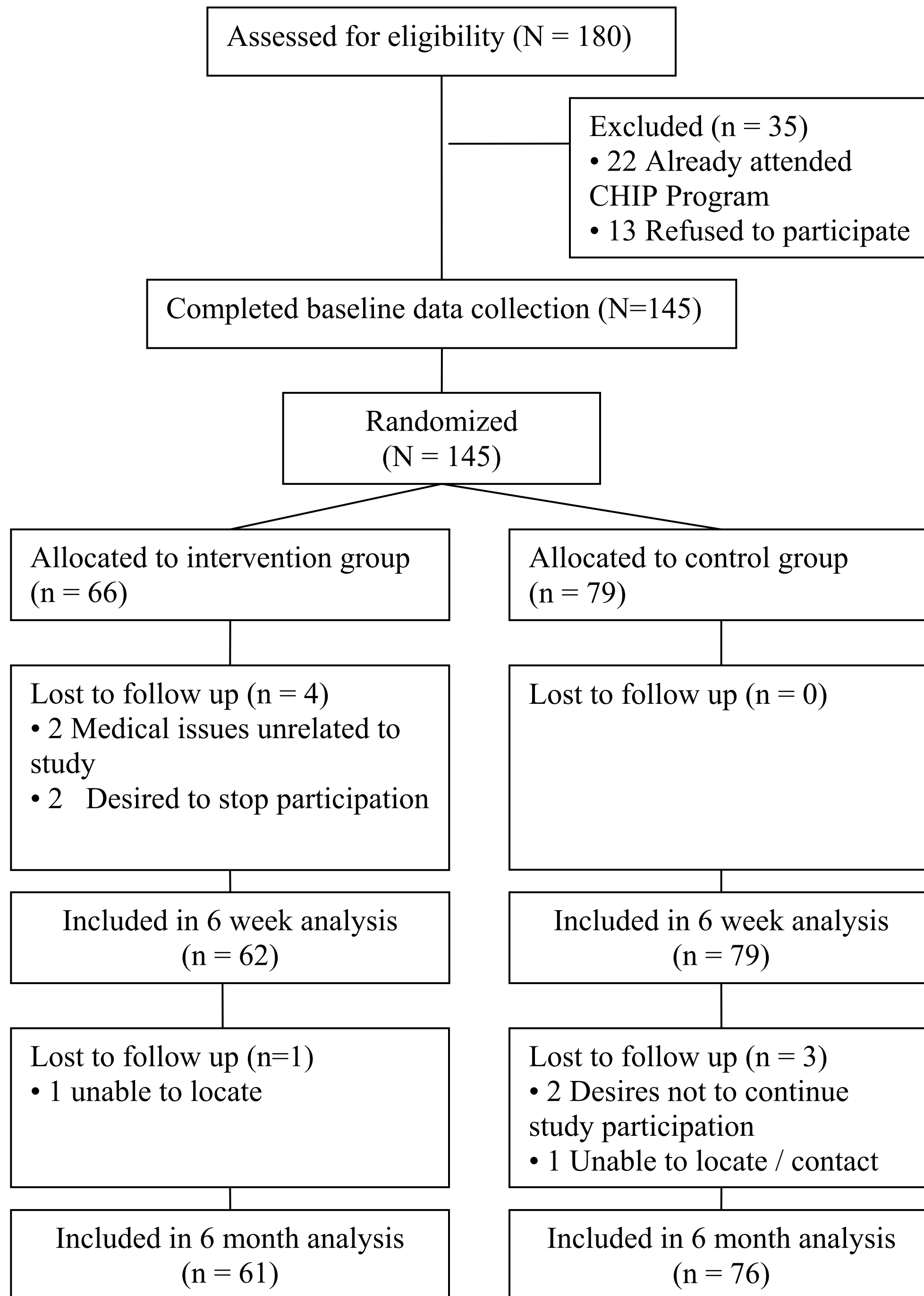

Table 1. Mean and Frequency Distributions for Intervention and Control Groups According to Selected Demographic Variables

	Intervention		Control		χ^2
	No.	%	No.	%	P Value
Gender					
Male	9	14.1	11	13.9	<0.001
Female	55	85.9	68	86.1	0.981
Race					
White alone	62	96.9	74	96.1	
Black alone	1	1.6	2	2.6	0.195
Other	1	1.6	1	1.3	0.907
Marital Status					
Never Married	5	7.8	9	11.4	
Married	50	78.1	59	74.7	
Divorced	6	9.4	10	12.7	2.338
Widowed	3	4.7	1	1.3	0.505
Annual Family Income					
$0–$20,000	4	6.4	2	2.5	
$20,001–$40,000	9	14.5	14	17.7	
$40,001–$60,000	13	21.0	20	25.3	1.836
$60,000 +	36	58.1	43	54.4	0.607
Education					
< High School	1	1.6	4	5.1	
High School	10	15.6	12	15.4	
Some College	27	42.2	26	33.3	
College Degree	19	29.7	17	21.8	6.332
Post College Degree	7	10.9	19	24.4	0.176

Table 2. Mean Change Scores for Intervention and Control Groups through 6 Weeks and 6 Months among Swedish American Employees According to Physical Activity and Nutrition Variables

	Intervention Group (n = 64)			Control Group (n = 79)			Between Group Δ Scores T-test P-value		Wilks' Lambda F-test P-value
	Baseline	Δ 6 Weeks	Δ 6 Months	Baseline	Δ 6 Weeks	Δ 6 Months	6 Weeks	6 Months	Time by Group
Total steps per week	47172.4	11607.4	7505.9	48273.8	-89.9	2132.5	0.0003	0.0803	0.0017
Total energy	2093.0	-266.8	-580.3	1793.3	-136.0	-119.7	0.2435	0.0004	0.0011
Calories from fat, %	34.5	27.5	27.8	34.3	33.2	35.6	<0.0001	<0.0001	<0.0001
Calories from protein, %	15.1	-0.8	-0.7	14.8	0.1	0.7	0.0337	0.0013	0.0048
Carbohydrates, %	51.5	9.6	8.6	51.2	1.1	-1.9	<0.0001	<0.0001	<0.0001
Fruit & vegetable fiber g	7.6	5.6	3.6	8.0	0.2	-0.3	<0.0001	<0.0001	<0.0001
Vegetable servings	3.2	1.6	1.5	3.3	0.0	0.1	<0.0001	0.0002	<0.0001
Fruit servings	1.4	1.3	0.6	1.5	0.1	0.0	<0.0001	<0.0001	<0.0001
Whole grain serving	6.0	1.0	0.0	4.9	-0.4	-0.6	0.0010	0.1358	0.0027
Meat servings	1.9	-0.3	-0.7	1.7	-0.1	0.0	0.4316	<0.0001	<0.0001
Total dietary fat g	83.5	-23.1	-34.6	71.1	-7.9	-2.6	0.0107	<0.0001	<0.0001
Dietary cholesterol, mg	205.2	-70.2	-110.4	161.6	-7.6	15.4	0.0006	<0.0001	<0.0001
Polyunsaturated fat, g	19.8	-4.1	-6.9	17.5	-2.0	-1.1	0.1652	0.0003	0.0006
Monounsaturated fat, g	32.0	-9.4	-13.7	27.6	-3.3	-0.9	0.0113	<0.0001	<0.0001
Saturated fat, g	25.0	-8.4	-12.1	20.5	-2.4	-0.8	0.0009	<0.0001	<0.0001
Sodium, mg	3003.0	-209.3	-689.3	2521.7	-184.1	-197.9	0.8824	0.0097	0.0202

Table 3. Mean Change Scores for Intervention and Control Groups through 6 Weeks and 6 Months among Swedish American Employees According to Cardiovascular Risk Factors Variables

	Intervention Group (n = 64)			Control Group (n = 79)			Between Group Δ Scores T-test P-value		Wilks' Lambda F-test P-value
	Baseline	Δ 6 Weeks	Δ 6 Months	Baseline	Δ 6 Weeks	Δ 6 Months	6 Weeks	6 Months	Time by Group
Body Mass Index	32.1	-1.1	-1.6	31.3	-0.2	-.03	<0.0001	<0.0001	<0.0001
Weight, kg	89.3	-2.9	-4.4	85.9	-0.4	-1.0	<0.0001	<0.0001	<0.0001
Body fat, %	40.0	-1.1	-2.4	38.4	-0.3	-0.4	0.0202	0.0008	0.0033
SBP, mm Hg	126.5	-7.2	-5.9	124.6	-5.4	-3.9	0.3028	0.3050	0.5071
DBP, mm Hg	77.6	-4.9	-6.5	75.6	-2.6	-3.8	0.0819	0.0506	0.1082
Resting heart rate, bts/min	73.4	-4.6	-4.7	72.4	-0.1	-1.8	0.0111	0.0729	0.0471
Glucose, mg/dL	97.6	-3.9	-2.0	99.7	-3.2	-2.0	0.7129	1.0000	0.8615
Cholesterol, mg/dL	199.6	-16.0	0.8	185.8	10.4	13.7	<0.0001	0.0153	<0.0001
HDL, mg/dL	45.8	-3.1	0.3	45.2	4.2	4.4	<0.0001	0.0006	<0.0001
LDL, mg/dL	128.4	-12.3	2.6	120.4	7.2	9.7	<0.0001	0.1237	<0.0001
Triglycerides, mg/dL	126.3	-3.2	-9.6	100.7	-4.7	-1.2	0.8599	0.4411	0.4239
hs-CRP, mg/dL†	3.79	-0.1	-0.2	3.6	-0.6	-0.5	0.3045	0.6006	0.6348

SBP = systolic blood pressure, DBP=diastolic blood pressure, HDL= high density lipoprotein, LDL= low density lipoprotein, hs-CRP = high sensitivity C-reactive protein.

Current Research

Effects of an Intensive Diet and Physical Activity Modification Program on the Health Risks of Adults

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ABSTRACT

Background This study assessed the clinical impact of lifestyle change education on chronic disease risk factors within a community.

Design Randomized clinical trial.

Setting/Participants Participants included 337 volunteers age 43 to 81 years from the Rockford, IL, metropolitan area.

Intervention The intervention group attended a 40-hour educational course delivered over a 4-week period. Participants learned the importance of making healthful lifestyle choices and how to make improvements in nutrition and physical activity.

Main Outcome Measures Changes in health knowledge, nutrition, and physical activity behavior, and several chronic disease risk factors were assessed at baseline and 6 weeks.

Results Beneficial mean changes in scores tended to be significant for the intervention group but not for the control group. Variables with improved scores included health knowledge, percent body fat, total steps per week, and most nutrition variables. Clinical improvements were seen in resting heart rate, total cholesterol, low-density lipoprotein cholesterol, and systolic and diastolic blood pressure. The control group experienced comparatively small but significant improvements in health knowledge, systolic and diastolic blood pressure, glucose, and in some nutrition variables. For almost all variables, the intervention group showed significantly greater improvements.

Conclusions This lifestyle modification program is an efficacious nutrition and physical activity intervention in the short term and has the potential to dramatically reduce the risks associated with common chronic diseases in the long term.

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Chronic diseases, such as cancer, cardiovascular disease, stroke, and diabetes, are responsible for most deaths in the United States (1). Approximately 70% to 90% of these deaths are estimated to be caused by poor nutrition, sedentary living, and tobacco use and are largely preventable (2-4). Despite remarkable pharmacological and technological advances, the greatest improvements in public health in the United States will be made by helping individuals adopt and maintain more healthful lifestyles. This would include avoidance of tobacco, healthful eating, and more consistent physical activity. Such lifestyles are causally linked to the prevention and arrest of cardiovascular diseases, diabetes, and to certain adult cancers. Yet, within the United States, 23% of adults smoke, 77% fail to consume a healthful diet, and 78% are at elevated health risk because they do not get enough physical activity (5).

In an effort to move beyond the treatment-centered medical care model, a progressive health care provider in Rockford, IL (SwedishAmerican Health System) has actively supported a variety of lifestyle intervention programs not traditionally found in health care settings. These include the Dr Dean Ornish Program for Reversing Heart Disease and the Coronary Health Improvement Project (CHIP). Findings from the Ornish program in Rockford have demonstrated that patients with coronary disease can markedly improve their nutritional and physical activity profiles for at least 1 year. This, in turn, dramatically reduced their coronary risk (6,7).

CHIP is designed for adults in the community. Its intent is to reduce chronic disease incidence and to improve the overall health of the public by providing a lifestyle change program within a community setting. The program highlights the importance of making more healthful lifestyle choices for preventing, arresting, and reversing many diseases common among people of industrialized nations and teaches participants how to implement these

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choices through a change in diet, physical activity, and smoking cessation. Two evaluations of CHIP using one-group pretest/posttest analysis showed that participants were able to significantly reduce blood pressure, body weight, and total and low-density lipoprotein (LDL) cholesterol within 4 weeks (8,9). These descriptive studies have demonstrated that the program seemed to help reduce not only coronary risk factors, but also reduce the risks associated with cancer, diabetes, and the metabolic syndrome.

Later, a videotaped version of the program was offered to 453 employees at six geographically different worksites. Research from SwedishAmerican Health System on the effect of the videos mirrored previous reports. Participants experienced significantly reduced chronic disease health risks (10). None of these published evaluations, however, used randomization or a control group.

Despite remarkable pharmacological and technological advances, the greatest improvements in public health in the United States will be made by helping individuals adopt and maintain more healthful lifestyles.

Using CHIP as the intervention, SwedishAmerican Health System initiated a large randomized clinical trial in March 2003 to answer the following questions: Do program participants improve their cognitive understanding of the importance of healthful lifestyles? Do participants improve their nutrition and physical activity behavior? Do participants experience significant improvements in a variety of factors associated with diabetes, hypertension, cardiovascular disease, and cancer? The trial is scheduled to be completed in the fall of 2005. This paper reports on the initial short-term results of the trial as it relates to these questions.

METHODS

Subject Recruitment and Design

The SwedishAmerican Center for Complementary Medicine recruited study subjects using targeted advertising, marketed through the Centers of Excellence, CHIP alumni groups, corporate client sites, and the SwedishAmerican Medical Group. Recruitment efforts targeted adults in the greater Rockford, IL, metropolitan area. The Figure shows the participant progress through the study. To be included, each participant had to be willing to begin the program starting in 1 month or in 7 months, and be at least 18 years of age. Eligible and interested participants provided informed consent. Potential participants were excluded if they had any significant systemic or major illnesses, including congestive heart failure, coronary artery disease, cerebrovascular disease, pulmonary disease with hypoxia, renal failure, organ transplantation, serious psychiatric disease, malignancy that (in the opinion of the investigators) would preclude adequate follow-up, or any other condition that, in the opinion of the investigators, would impede participation in regular physical activity.

Participants were highly encouraged to participate with a spouse or partner. For couples who agreed to participate, the unit of randomization was the couple. For individuals who wished to participate, the unit of randomization was the individual. Final enrollment included 58 pairs evenly distributed across the treatment and control group. The unit of analysis in the statistical evaluation was individuals. By completing the 6-week data collection follow-up participants became eligible to receive a $50 incentive. The allocation sequence was created using a random number generator. Program sign-up and randomization to groups were made by the study coordinator. The study was approved by the Institutional Review Board of SwedishAmerican Health System, and is in compliance with the Health Insurance Portability and Accountability Act guidelines.

Intervention

The intervention consisted of the live lecture version of CHIP (8,9). Participants met for 4 weeks—four times each week for 2 hours each time—when they received instruction. The curriculum topics covered modern medicine and health myths, atherosclerosis, coronary risk factors, obesity, dietary fiber, dietary fat, diabetes, hypertension, cholesterol, exercise, osteoporosis, cancer, lifestyle and health, the Optimal Diet, behavioral change (8), and self-worth.

In conjunction with the lectures, participants received workbooks that closely follow the discussion topics and contain assignments with learning objectives for each topic. These assignments were designed to help in the understanding and integration of the concepts and information presented. In addition, dietetics professionals and medical professionals spoke to the group weekly about the prevention, arrest, and reversibility of chronic diseases. Participants had access to scheduled shopping tours at local supermarkets and cooking demonstrations given by a dietetics professional.

The presenter of the educational sessions was available to answer questions regarding the presentations, workbook assignments, and the program, with assistance of program staff. Participants were encouraged to follow preset dietary and exercise goals. The dietary goal involved adopting a more plant-based diet that emphasizes largely unrefined "food-as-grown." These foods (such as grains, legumes, fresh fruits, and vegetables), usually high in unrefined complex carbohydrates, were encouraged. A whole-food diet—low in fat, animal protein, sugar, and salt; high in fiber, antioxidants, and micronutrients; and very low in cholesterol—is in contrast to the typical Western diet. Participants were encouraged to eat some nuts and were introduced to the idea of topping cereal with ground flax seed. At the same time, program participants were encouraged to progressively implement an exercise program (30 min/day) of walking and general fitness. At the completion of the program, participants were encouraged to join the Rockford CHIP Alumni Organization for a cost of $35 per year. The group receives a monthly newsletter, which contains news of health-promoting community events such as healthful dinners, walking groups, and support group meetings. The alumni

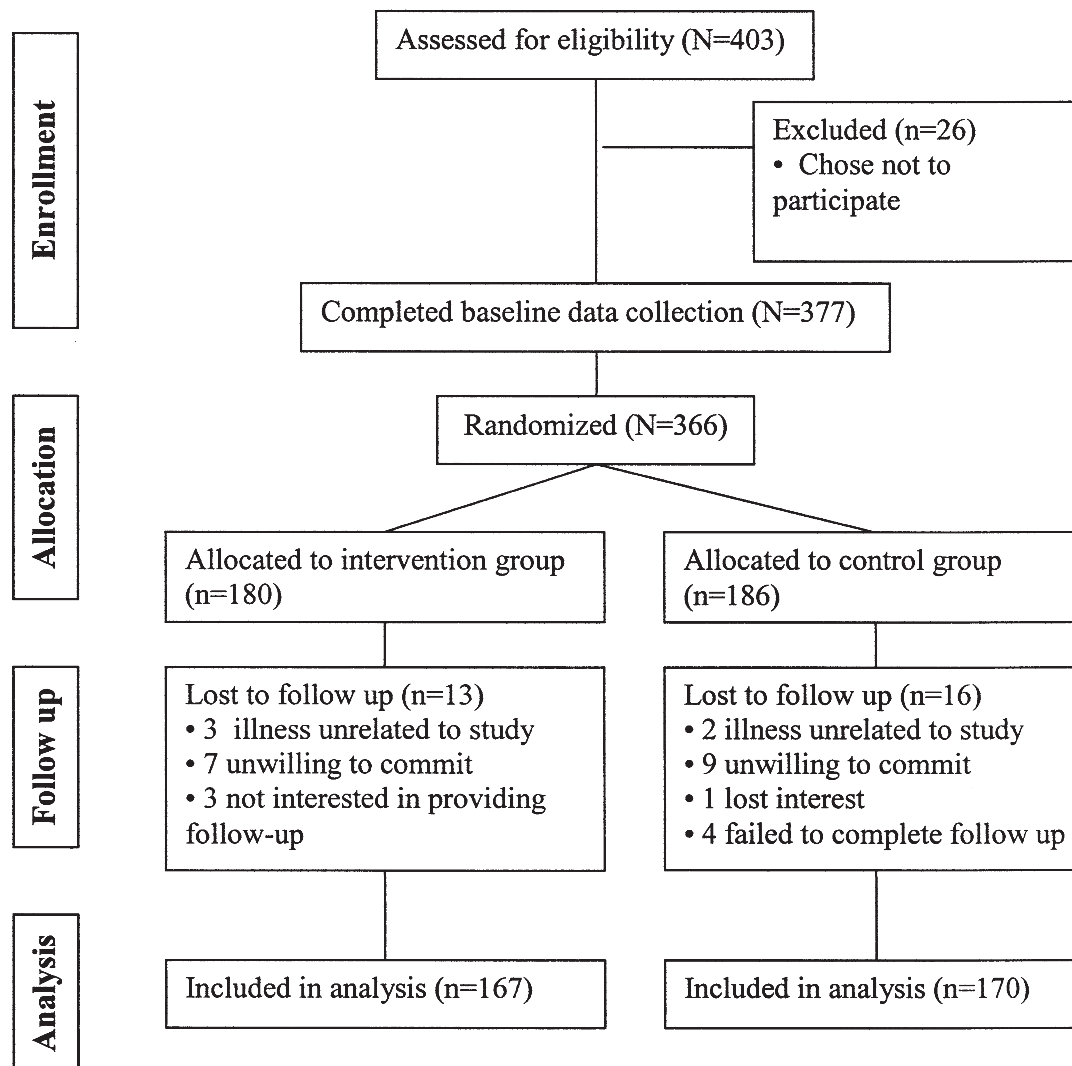

Figure. Flow of participants through each stage of study of an intensive diet and physical activity program on the health risks of adults.

are encouraged to attend special lectures on healthful living and relapse prevention.

Participants assigned to the control group were screened at baseline and told they would be able to participate in the program in 6 months. They were again screened after 6 weeks.

Measures

Data collection personnel were blinded to group assignment. Data gathered included demographic, cognitive, and behavioral measurements, and physiological outcomes related to chronic disease. Demographic data were collected at baseline. A first step in behavior change may involve increasing awareness of proper health behaviors and knowledge. Specific knowledge regarding health risks, proper eating and physical activity behaviors, and an understanding of the benefits of a healthful lifestyle were assessed with a multiple-choice health knowledge test. The test has previously demonstrated validity and reliability (8). Information about each of the 30 questions is contained in the textbook, handout materials, and in a CHIP video lending library, so that participants who missed a lecture could obtain the information. The number of correct responses was recorded. Attendance at each of the classes was tracked.

The intervention was designed to assist individuals in adopting healthful eating and physical activity behaviors. To assess dietary intake, the Block 98 full-length dietary questionnaire (Block 98.2, 1998, Block Dietary Data Systems, Berkeley, CA) was used. It was originally developed with the National Cancer Institute for research into the role of diet in health and disease and has been continually updated and improved. The questionnaire has been extensively studied and validated (11-13). It is self-reported and optically scanned and scored. The variables measured by this survey include, but are not limited to, the

following: daily nutrients from food, percent of energy from different nutrients, fiber from different sources, and food group servings per day.

To ascertain energy expenditure contributed by physical activity, a 7-day self-recorded pedometer log was maintained by each participant. Participants wore the Walk4Life Model 2000 Life Stepper pedometer (Walk4Life Inc, Plainfield, IL) on a belt at the right hip directly above the right kneecap each day for 7 days. Immediately before going to bed the pedometer counts for the day were recorded and the number reset. Strike counts from pedometers are a valid and reliable method of monitoring and measuring free-living physical activity (14-16).

The main outcome variables for this study included several chronic disease risk factors. After a 12-hour fast, blood was drawn using a vacutainer (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) by phlebotomists from SwedishAmerican Health System's outpatient laboratory. Samples were allowed to clot and then centrifuged. Clinical analyses were completed at the SwedishAmerican Health System laboratory. Lipid analysis followed the lipid standards provided by the Centers for Disease Control and Prevention. Total cholesterol, high-density lipoprotein (HDL), and triglyceride concentrations were determined using Beckman-Coulter instrumentation and methodology. LDL values were calculated as follows: LDL=total cholesterol−HDL−(triglycerides/5) (17). High-sensitivity C-reactive protein was determined using a microplate protocol based on a latex bead enhanced immunoturbidity assay (18,19). Glucose was determined using a Kodak Ektachem (Eastman Kodak, Rochester, NY). Trained program staff took blood pressure measures; after resting for 5 minutes, blood pressure was measured using the guidelines and protocol set forth by the American Heart Association (20). Weight and height were measured using standard medical weight and height scales recently calibrated by the Biometrics Department of SwedishAmerican Health System. Percent body fat was estimated using the Tanita TBF-300A electrical impedance scale (Tanita Corp, Arlington Heights, IL) (21). Any participants who were high risk for any of the risk factors measured were encouraged to see their physician.

Statistical Analyses

Cross-tabulations were used to perform bivariate analyses between selected variables, with statistical significance based on the χ^2 test for independence (22). The t test method was used for testing differences in means (23). Risk factor cut points (Tables 1 and 2) were previously established (24,25) and categorized accordingly. Analyses were performed using SAS version 8.2 (SAS Institute Inc, Cary, NC, 2001). Procedure statements used in SAS for assessing the data were PROC UNIVARIATE, PROC FREQ, PROC TTEST, and PROC GLM.

Because multiple pair-wise tests were performed, an adjusted α should be referred to in order to minimize the overall probability of committing a type 1 error. The modified α based on the Bonferroni correction (27), pair-wise tests, and α=.05 is .0001. This conservative α should be used when determining significance in Tables 1 and 2. For all other results, statistical significance was based on the .05 level.

RESULTS

Analyses are based on 337 participants who completed both baseline and 6-week data screenings. Of the 366 participants that were randomized, 29 were lost to follow-up (Figure). Dropout rates between groups were similar. A description of participants in the intervention and control groups is presented according to age, sex, race, marital status, income, and education in Table 3. There were no statistically significant differences between participants in the intervention and control groups for these variables. Ages ranged from 43 to 81 years. The majority of participants were female, white, married, had an annual family income more than $40,000, and had at least some college education. Program attendance from the intervention group averaged 79% across all lecture sessions.

Baseline means and mean change scores are presented according to group assignment in Table 4. The mean change in scores within groups through 6 weeks tended to be significant for the intervention group but not for the control group. This included the following variables: total steps per week, resting heart rate, weight, percent body fat, percentage of energy from fat and carbohydrates, fiber from vegetables and fruits, vegetable servings, fruit servings, grain servings, meat servings, and dietary cholesterol. For several variables, the change was significant for both groups: health knowledge, energy, total dietary fat, saturated fat, polyunsaturated fat, monounsaturated fat, sodium, systolic and diastolic blood pressure, glucose, serum cholesterol, HDL, and LDL. Mean change in scores through 6 weeks was not significant for either intervention or control for triglycerides and C-reactive protein. We also considered whether the change scores were significantly different between intervention and control groups for each variable. Significantly greater changes were experienced by the intervention group for all variables except sodium, triglycerides, and C-reactive protein. The control group had significantly higher total cholesterol, LDL, and HDL at 6 weeks.

Mean baseline, 6-week, and change scores are presented according to standard health risk cut points for the risk factor variables and intervention (Table 1) and control status (Table 2). This analysis stratifies results according to risk status. Individuals with low risk would not be expected to experience large changes, but risk values considered to be high would be expected to change significantly. For the intervention group, the distributions favorably changed between baseline and 6 weeks for systolic and diastolic blood pressure, total cholesterol, LDL cholesterol, and fruit and vegetable consumption. Corresponding significant changes in the distributions between baseline and 6 weeks were not observed in the control group. Mean change scores within baseline risk categories tended to be significant for both intervention and control groups. They were, however, more significant in the intervention group and the favorable changes in risk behaviors were generally higher for those in the intervention group.

DISCUSSION

Findings from this study indicate that lifestyle change can result in significant short-term improvements in

Table 1. Health risk prevalence and baseline means, follow-up means, and change scores through 6 weeks for the intervention group

	Baseline		6 Weeks		χ^2 (trend) *P* value[a]	Baseline mean	Follow-up mean[b]	Mean change	T statistic *P* value
	n	%	n	%					
BMI[c]									
Underweight (<18.5)	0	0.00	0	0.00	.3066	—	—	—	—
Normal (18.5-24.9)	24	14.37	29	17.37		22.74	22.00	−0.74	<.0001
Overweight (25.0-29.9)	43	25.75	47	28.14		27.72	26.63	−1.09	<.0001
Obese (≥30.0)	100	59.88	91	54.49		38.31	36.91	−1.40	<.0001
Systolic BP[d] (mm Hg)									
Normal (<120)	49	29.34	79	47.31	.0003	111.08	109.15	−1.93	.2458
Prehypertensive (120-139)	78	46.71	67	40.12		129.31	122.14	−7.17	<.0001
High (140-159)	32	19.16	17	10.18		147.77	134.56	−13.21	<.0001
Dangerous (≥160)	8	4.79	4	2.40		167.50	152.12	−15.38	.0001
Diastolic BP (mm Hg)									
Normal (<80)	92	55.09	127	76.05	<.0001	71.90	69.67	−2.23	.0046
Prehypertensive (80-89)	53	31.74	36	21.56		83.45	77.00	−6.45	<.0001
High (90-99)	20	11.98	3	1.80		93.25	79.20	−14.05	<.0001
Dangerous (≥100)	2	1.20	1	0.60		—	—	—	—
Total CHOL[e] (mg/dL)									
Normal (<200)	99	59.28	135	80.84	<.0001	172.0	166.0	−6.0	.0144
Borderline (200-239)	19	11.38	12	7.19		214.8	191.6	−23.2	<.0001
High risk (≥240)	49	29.34	20	11.98		257.3	214.2	−43.1	<.0001
LDL[f] (mg/dL)									
Optimal (<100)	42	25.15	56	33.53	.0102	85.02	87.90	2.88	.6289
Above optimal (100-129)	67	40.12	73	43.71		115.76	107.96	−7.80	.0019
Borderline (130-159)	42	25.15	30	17.96		142.57	123.21	−19.36	<.0001
High (160-189)	14	8.38	7	4.19		172.64	133.93	−38.71	<.0001
Very high (≥190)	2	1.20	1	0.60		—	—	—	—
HDL[g] (mg/dL)									
High (≥60)	17	10.18	15	8.98	.2716	70.00	61.29	−8.71	<.0001
Normal (40-59)	82	49.10	73	43.71		48.38	44.34	−4.04	<.0001
Low (<40)	68	40.72	79	47.31		34.48	33.50	0.98	.2222
Triglycerides[h] (mg/dL)									
Normal (<150)	119	71.26	115	68.86	.6749	88.96	103.75	14.79	.0026
Borderline (150-199)	26	15.57	30	17.96		171.23	158.38	−12.85	.2084
High (200-499)	20	11.98	21	12.57		286.35	213.55	−72.80	<.0001
Very high (≥500)	1	1.20	1	0.60		—	—	—	—
Glucose (mg/dL)									
Normal (<110)	132	79.04	137	82.04	.2686	93.72	92.35	−1.37	.3022
IFG[i] (110-125)	14	8.38	17	10.18		113.93	106.79	−7.14	.0691
Diabetes (≥126)	21	12.57	13	7.78		152.00	132.52	−19.48	<.0001
Fruit and vegetable (servings/d)									
Low (<5/day)	108	64.67	47	28.14	<.0001	3.20	6.66	3.46	<.0001
Healthy (≥5/day)	59	35.33	120	71.86		7.74	9.17	1.43	.0015

[a]Mantel-Haenszel χ^2 test.
[b]Follow-up means are from the same individuals in each baseline risk category.
[c]BMI=body mass index.
[d]BP=blood pressure.
[e]CHOL=cholesterol.
[f]LDL=low-density lipoprotein.
[g]HDL=high-density lipoprotein.
[h]n does not equal 167 due to missing data point.
[i]IFG=impaired fasting glucose.

health knowledge, nutrition, and physical activity behavior, and significant improvements in many chronic disease risk factors. Significant improvements in health knowledge among the intervention group were concurrent with improvements in nutrition and physical activity. The number of participants who consumed five servings of fruits and/or vegetables per day doubled as the average participant doubled the number of servings con-

Table 2. Health risk prevalence and baseline means, follow-up means, and change scores through 6 weeks for the control group

	Baseline		6 Weeks		χ^2 (trend) *P* value[a]	Baseline mean	Follow-up mean[b]	Mean change	T statistic *P* value
	n	%	n	%					
BMI[c]									
Underweight (<18.5)	1	0.59	1	0.59	≈1.0000	—	—	—	—
Normal (18.5-24.9)	43	25.29	45	26.47		23.15	23.06	−0.09	.3680
Overweight (25.0-29.9)	54	31.76	50	29.41		27.41	27.14	−0.27	.0035
Obese (≥30.0)	72	42.35	74	43.53		38.44	38.38	−0.06	.4543
Systolic BP[d] (mm Hg)									
Normal (<120)	60	35.29	81	47.65	.0656	111.07	109.92	−1.15	.4245
Prehypertensive (120-139)	66	38.82	55	32.35		128.73	123.23	−5.50	<.0001
High (140-159)	39	22.94	27	15.88		149.50	143.56	−5.94	.0011
Dangerous (≥160)	5	2.94	7	4.12		165.40	144.60	−20.80	<.0001
Diastolic BP (mm Hg)									
Normal (<80)	100	58.82	115	67.65	.1514	70.47	69.60	−0.87	.2811
Prehypertensive (80-89)	50	29.41	40	23.53		82.12	78.44	−3.68	.0015
High (90-99)	18	10.59	12	7.06		93.33	86.94	−6.39	.0009
Dangerous (≥100)	2	1.18	3	1.76		—	—	—	—
Total CHOL[e] (mg/dL)									
Normal (<200)	112	65.88	123	72.35	.3137	164.6	179.8	15.2	<.0001
Borderline (200-239)	22	12.94	15	8.82		219.8	222.8	3.0	.4284
High risk (≥240)	36	21.18	32	18.82		259.9	247.1	−12.8	.0611
LDL[f] (mg/dL)									
Optimal (<100)	46	27.06	34	20.00	.1255	82.02	93.98	11.96	.0072
Above optimal (100-129)	57	33.53	62	36.47		113.54	127.30	13.76	<.0001
Borderline (130-159)	46	27.06	45	26.47		142.67	144.89	2.22	.5381
High (160-189)	17	10.00	22	12.94		170.35	166.76	−3.59	.5020
Very high (≥190)	4	2.35	7	4.12		—	—	—	—
HDL[g] (mg/dL)									
High (≥60)	16	9.41	25	14.71	.0565	65.06	61.56	−3.5	.0450
Normal (40-59)	96	56.47	100	58.82		48.16	51.25	3.09	<.0001
Low (<40)	58	34.12	45	26.47		34.02	39.12	5.10	<.0001
Triglycerides (mg/dL)									
Normal (<150)	136	80.00	133	78.24	≈1.0000	83.56	90.65	7.09	.0738
Borderline (150-199)	17	10.00	23	13.53		171.94	171.38	−0.56	.9645
High (200-499)	16	9.41	13	7.65		262.75	215.94	−46.81	.0003
Very high (≥500)	1	0.59	1	0.59		—	—	—	—
Glucose (mg/dL)									
Normal (<110)	149	87.65	145	85.29	.5100	94.64	93.44	−1.2	.1658
IFG[h] (110-125)	14	8.24	16	9.41		113.21	109.93	3.28	.3272
Diabetes (≥126)	7	4.12	9	5.29		175.57	155.43	20.14	<.0001
Fruit and vegetable (servings/d)									
Low (<5/day)	105	61.76	96	56.47	.3215	3.22	3.78	0.56	.0077
Healthy (≥5/day)	65	38.24	74	43.53		7.82	7.28	−0.54	.0460

[a]Mantel-Haenszel χ^2 test.
[b]Follow-up means are from the same individuals in each baseline risk category.
[c]BMI=body mass index.
[d]BP=blood pressure.
[e]CHOL=cholesterol.
[f]LDL=low-density lipoprotein.
[g]HDL=high-density lipoprotein.
[h]IFG=impaired fasting glucose.

Table 3. Mean and frequency distributions for selected demographic variables according to intervention- and control-group status

	Intervention[a]		Control[b]		T statistic	P value
Age	50.4±11.1[c]		50.8±11.1[c]		−0.35	.7149
	n	%	n	%	χ^2	
Sex					0.15	.7008
Male	45	27.0	49	28.8		
Female	122	73.1	121	71.2		
Race					4.04	.1323
White	161	96.4	156	92.9		
Black	3	1.8	10	6.0		
Other	3	1.8	2	1.2		
Marital status					3.40	.3345
Never married	12	7.2	20	11.8		
Married	133	80.1	123	72.8		
Divorced	15	9.0	16	9.5		
Widowed	6	3.6	10	5.9		
Annual family income ($)					1.74	.6268
0-20,000	13	8.0	12	7.2		
20,001-40,000	35	21.5	27	16.3		
40,001-60,000	34	20.9	40	24.1		
>60,000+	81	49.7	87	52.4		
Education					7.36	.1179
< High school	6	3.6	7	4.1		
High school	34	20.4	45	26.5		
Some college	58	34.7	38	22.4		
College degree	37	22.2	37	21.8		
Post college degree	21	19.2	43	25.3		

[a]n may not equal 167 due to missing data points.
[b]n may not equal 170 due to missing data points.
[c]Mean±standard deviation.

sumed per day, leaving only 24% of the intervention group still eating fewer than five servings a day at follow-up. This is in contrast to Behavior Risk Factor Surveillance data from the state of Illinois, which shows that 79% of Illinoisans fail to get five servings a day (5).

Pedometer data show that the program participants increased pedometer monitored movement by 30%. This improvement in physical activity is corroborated with a significant decrease in resting heart rate, a correlated measure of cardiorespiratory fitness (26). Increases in physical activity can increase heart size, blood volume, stroke volume, and cardiac output (27).

Poor nutrition and sedentary living are associated with a constellation of risk factors, some of which have been identified in the metabolic syndrome, and all of which are known to be linked to diabetes, cardiovascular disease, and cancer. Improvements in nutrition and physical activity are associated with significant improvements in diabetes risk as whole body glucose tolerance improves, insulin sensitivity increases, and the amount of glucose transporter (GLUT4) increases (28). The Glucose section of Table 1 shows that the number of individuals in the intervention group who were diabetic (glucose≥126)* at baseline was reduced from 21 to 13, representing a 38% reduction in diabetes prevalence and a significant reduction in fasting blood glucose. The reductions in fasting blood glucose, body fat, and body weight reported here are similar to improvements reported from other evaluations of the program (8-10).

Lower blood pressure, improved blood lipids, and improved cardiac function are directly linked to reduced risk of cardiovascular disease. For every 1% drop in total cholesterol, the coronary risk drops by 2% to 3%; and for every 1 mm Hg drop in elevated diastolic blood pressure, there is another 2% to 3% drop in coronary risk (29). On average, the net decrease (between intervention and control group) was 3 mm Hg for diastolic blood pressure and 12% for total cholesterol. When only those with elevated baseline diastolic blood pressure (90 to 99 mm Hg) or cholesterol (>200 mg/dL)† were considered, then the net reductions were 7 mm Hg and 27 mg/dL (12.2% cholesterol reduction), respectively. Interventions with hypercholesterolemic patients using the National Cholesterol Education Program's step 1 and step 2 dietary interventions reported,

**To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0. To convert mg/dL glucose to mmol/L, multiply mg/dL by 0.0555. Glucose of 6.0 mmol/L=108 mg/dL.*

†To convert mmol/L cholesterol to mg/dL, multiply mmol/L by 38.7. To convert mg/dL cholesterol to mmol/L, multiply mg/dL by 0.026. Cholesterol of 5.00 mmol/L=193 mg/dL.

Table 4. Mean baseline scores and mean change in scores through 6 weeks by intervention and control groups

Variable	No.	Baseline		6 Weeks		T statistic
		Mean	Standard deviation	Mean change	Standard deviation	*P* value[a]
Health knowledge***						
Intervention	167	15.95	3.54	7.92	5.01	<.0001
Control	170	14.84	3.26	0.85	3.21	.0087
Total steps***						
Intervention	167	40,583	22,777	12,080	16,909	<.0001
Control	170	44,136	23,545	2,057	15,936	.1044
% of energy from fat***						
Intervention	163	36.26	6.59	−7.95	10.64	<.0001
Control	168	34.65	6.68	−0.64	5.36	.3257
% Carbohydrates***						
Intervention	163	49.26	8.39	10.57	12.54	<.0001
Control	168	51.01	8.07	0.51	6.12	.4994
Fruit and vegetable fiber (g)***						
Intervention	163	7.96	4.42	5.41	6.62	<.0001
Control	168	8.36	4.98	0.21	3.66	.6175
Vegetable servings***						
Intervention	163	3.37	2.14	1.58	2.60	<.0001
Control	168	3.42	2.20	0.03	1.80	.8735
Fruit servings***						
Intervention	163	1.47	1.07	1.15	1.46	<.0001
Control	168	1.58	1.05	0.11	0.87	.2162
Whole-grain servings***						
Intervention	163	5.52	3.08	1.04	2.93	<.0001
Control	168	5.04	2.44	−0.33	1.82	.0773
Meat servings*						
Intervention	163	2.07	1.40	−0.48	1.39	<.0001
Control	168	1.89	1.19	−0.10	1.12	.3114
Total dietary fat (g)***						
Intervention	163	87.02	55.58	−28.93	51.64	<.0001
Control	168	76.73	42.48	−7.83	29.03	.0155
Dietary cholesterol (mg)***						
Intervention	163	209.60	143.09	−84.02	157.16	<.0001
Control	168	179.00	109.01	−11.97	81.15	.2138
Polyunsaturated fat (g)**						
Intervention	163	20.93	14.20	−5.91	12.38	<.0001
Control	168	19.24	11.93	−1.79	8.60	.0305
Monounsaturated fat (g)***						
Intervention	163	33.67	21.61	−11.65	23.51	<.0001
Control	168	29.79	17.05	−3.23	13.46	.0144
Saturated fat (g)***						
Intervention	163	25.70	17.40	−9.97	16.54	<.0001
Control	168	21.78	12.01	−2.51	7.89	.0120
Sodium (mg)						
Intervention	163	2,921	1,524	−326	1181	<.0001
Control	168	2,700	1,223	−204	903	.0124
Calories						
Intervention	163	2,079	1,031	−350	859	<.0001
Control	168	1,917	802	−146	554	.0091
Weight (lb)***						
Intervention	167	205.44	53.56	−7.55	4.72	<.0001
Control	170	192.31	56.23	−0.29	8.51	.5817
Percent body fat***						
Intervention	163	36.26	7.31	−7.95	10.64	<.0001
Control	168	34.65	7.39	−0.64	5.36	.3257

Table 4. Mean baseline scores and mean change in scores through 6 weeks by intervention and control groups (continued)						
		Baseline		6 Weeks		
Variable	No.	Mean	Standard deviation	Mean change	Standard deviation	T statistic *P* value[a]
Systolic BP[b] (mm Hg)						
Intervention	167	129.33	16.37	−7.20	11.86	<.0001
Control	170	128.34	16.83	−4.52	11.59	<.0001
Diastolic BP (mm Hg)*						
Intervention	167	78.49	9.30	−5.04	8.11	<.0001
Control	170	76.68	9.67	−2.31	8.19	.0003
Resting heart rate (beats/min)						
Intervention	162	73.26	10.20	−2.83	10.32	.0009
Control	166	72.06	10.55	0.16	11.18	.8458
Glucose (mg/dL)						
Intervention	166	102.80	23.08	−4.11	15.74	.0003
Control	166	99.62	19.45	−2.40	13.00	.0335
Cholesterol (mg/dL)***						
Intervention	166	191.86	31.82	−14.43	28.22	<.0001
Control	166	189.93	38.14	8.86	26.50	<.0001
HDL[c] (mg/dL)***						
Intervention	166	44.98	12.14	−3.39	7.13	<.0001
Control	166	45.19	10.39	2.85	7.22	<.0001
LDL[d] (mg/dL)***						
Intervention	165	120.96	28.96	−11.22	22.95	<.0001
Control	166	121.70	32.60	6.03	24.30	.0012
Triglycerides (mg/dL)						
Intervention	166	133.28	102.19	0.35	59.24	.9513
Control	166	114.40	86.90	0.47	55.50	.9032
CRP[e] (mg/dL)						
Intervention	162	4.04	3.56	−0.28	2.88	.2407
Control	164	3.70	3.45	0.03	3.20	.9071

[a]Evaluating the change in means from baseline through 6 weeks within categories of group.
[b]BP=blood pressure.
[c]HDL=high-density lipoprotein.
[d]LDL=low-density lipoprotein.
[e]CRP=C-reactive protein.
*$P<.01$, based on the T statistic assessing difference in mean change scores between groups.
**$P<.001$, based on the T statistic assessing difference in mean change scores between groups.
***$P<.0001$, based on the T statistic assessing difference in mean change scores between groups.

on average, total cholesterol reductions of 10% and 13%, respectively (30,31).

Blood pressure changes among program participants were equally dramatic. The baseline prevalence of hypertension in the intervention group was 18.5%; at follow up the prevalence was reduced to 7.5%. The PREMIER Clinical trial used a 6-month comprehensive lifestyle modification trial to reduce blood pressure (32). Hypertension prevalence at baseline and 6 months in this study was 38% and 12%, respectively, providing further support that change in nutrition and physical activity can directly impact blood pressure, especially among those who are hypertensive. Medication monitoring revealed that 12 program participants reduced their blood pressure medication during the intervention, suggesting that the program could have produced reductions in blood pressure greater than what was measured.

Ideally, individuals who participate in lifestyle interventions would adopt and maintain healthful behaviors for life. In reality, once the lifestyle interventions are completed, many individuals eventually fail to completely embrace new lifestyle habits and revert to pretreatment behavior. This phenomenon could be called healthful lifestyle decay and is thought to begin sometime after the completion of a lifestyle intervention. The short-term nature of this study sheds little light on long-term adherence, but it does allow for an accurate assessment of the acute benefit of the intervention. If follow-up data had only been collected at 12 months, it is likely that the data could have missed the period of greatest behavior change and risk factor improvement.

The observed improvements in behavior and risk reported here are not unexpected. The participants were mostly white and sufficiently self motivated to volunteer to participate in the intervention. On average, participants were slightly more educated than the community average. Participants had lifestyles that permitted them to attend most, if not all, of the classes. This is evident in

the high rate of attendance to this time-intensive program. These delimitations do threaten the generalizability of these findings and make application of the intervention to other populations problematic. Because the participants were somewhat select, the results from this intervention may represent a best-case scenario.

Other factors may explain the impact of this program. In the intervention, participants attended highly interactive lectures structured around the health belief and transtheoretical models (33,34). Video clips, testimonials, role playing, short presentations from physicians, social support strategies, food selection and planning activities, and other behavior-change-driven pedagogical activities all helped to encourage participants to enthusiastically evaluate personal behaviors and commit to make changes. To prevent relapse and help participants maintain their new behaviors, the program graduates were invited to participate in the alumni program. This group received a monthly newsletter, held socials, dinners, and special events designed to help participants maintain their new behaviors.

Despite the observed efficacy of this intervention, shortcomings of the study warrant discussion. Both the physical activity and nutrition data were self-reported. Also, for some variables the control group experienced significant improvement. Small but significant decreases in energy intake, percent of energy from fat, and sodium intake were found as well as small reductions in blood pressure and glucose. It is plausible that members of the control group may have made some positive lifestyle changes as they anticipated their own program participation some 6 months later. Further confounding may have occurred in that they may have frequented some of the 30 restaurants in the Rockford area that offer healthful CHIP menu items.

Ideally, individuals who participate in lifestyle interventions would adopt and maintain healthful behaviors for life.

This intervention, with a free-living though self-selected population, is an efficacious behavior-change intervention in the short term. Large beneficial changes in health behavior and risk factor levels were observed. Until long-term evaluations are completed, it remains to be seen if these improvements can be maintained and if long-term chronic disease incidence can be reduced.

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