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Long-term exposure to incense smoke alters metabolism in Wistar albino rats

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The burning of incense is an important source of indoor air pollution in Asia. We assessed the effect of long-term exposure to incense smoke on the body weight and levels of circulating glucose, triglycerides, total cholesterol, HDL-cholesterol, insulin, adiponectin and leptin in Wistar albino rats. Two groups of rats were used. First group ($n = 12$) was exposed daily to incense smoke for 4 months at the rate of 4 g day^{-1} in the exposure chamber. Another group of rats ($n = 12$), was used as non-exposed control. Blood samples were collected from all animals after 4, 8, 12 and 16 weeks of exposure. Serum glucose, triglycerides, total cholesterol and HDL-cholesterol, LDL-cholesterol insulin, adiponectin and leptin were measured. Our results showed that incense smoke exposure was associated with decreased weight gain and the adverse metabolic changes of increased triglycerides and decreased HDL-cholesterol concentrations. Exposure to incense was also associated with a transient increase of leptin levels. Taken together, these data suggest that incense smoke influences metabolism adversely in rats. The effect of incense smoke on human health and the underlying mechanisms need to be studied further. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS — Wistar albino rats; incense; triglycerides; cholesterol; leptin

INTRODUCTION

Incense use is an integral part of daily life in large parts of Asia.[1] In Gulf countries, incense is one of the common indoor smoke sources to which individuals are frequently exposed.[2] The most frequently used incense for burning is oud. The tree referred to as 'oud' is *Aquilaria agallocha*, also known as lignum aloes, aloes wood, agarwood and eaglewood. Its unique aroma is due to a fungal infection, which causes the tree to secrete an aromatic protective resin that has been widely used in the Middle East as a source of incense and perfume. Other types of incense (such as bakhour) are derived from sandalwood and are usually mixed with other ingredients, such as agarwood, natural oils, and other natural substances. Frankincense is a resin produced by small pine like trees of the genus Boswellia that grow only in arid areas of southern Arabia and in parts of Somalia, Sudan and Ethiopia.[3]

Due to its slow and incomplete combustion, incense burning produces continuous smoke, generating pollutants, such as toxic gases and chemical particles, including polycyclic aromatic hydrocarbons, carbon monoxide, benzene and isoprene, that easily accumulate indoors, especially in the presence of inadequate ventilation.[4] Several researchers in Asia and North America have investigated possible links between incense stick exposure and health problems, including respiratory symptoms, asthma, elevated cord blood IgE levels, contact dermatitis and cancer.[4–6] In addition, exposure to incense induced significant morphological changes of rat pneumocytes.[7]

Adipocytokines, including, adiponectin and leptin, are the major adipose tissue-derived protein hormones, with multiple biological functions.[8] Adiponectin, an adipose tissue-specific collagen-like factor, is emerging as an important molecule in obesity,[9] the metabolic syndrome[10–12] and cardiovascular disease.[13] Leptin, a protein hormone secreted by white adipocytes, also serves as an endocrine signal involved in the regulation of appetite and energy expenditure.[14–16]

The purpose of this study was to assess the effects of long-term exposure to incense smoke on body weight and the levels of glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-Cholesterol, insulin, adiponectin and leptin in Wistar albino rats.

MATERIALS AND METHODS

Experimental animals

Wistar albino male rats (*Rattus norvegicus*) of approximately the same age, and weighing 290 ± 20 g were used under a protocol in accordance with guidelines and approved

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by the local Ethical Committee for the protection of animals used for experimental purposes. Animals were supplied from the King Saud University colony. Rats were maintained in air-conditioned rooms at 22 °C. They received food and water *ad libitum*.

Incense exposure

After a 2-week acclimatization period, the rats were randomly divided into two groups. Group 1 was exposed to incense smoke; group 2 was exposed to fresh air (control). Specifically, the animals in the incense group were exposed daily to incense smoke by burning 4 g of incense per day in an exposure chamber. Incense used was obtained from agarwood. The animals in the control group were continuously exposed to fresh air and subjected to exactly the same conditions of restraint, caging and nutrition as the exposed to incense burning group.

Samples analyses

At 4, 8, 12, 16-weeks after the onset of treatment, rats had a 12 h fast overnight. In the morning they were exposed to light anesthesia and had blood samples collected from their tail vein. Blood samples were centrifuged at 1200 rpm at 4 °C and the clear supernatant of serum was transferred to a fresh tube for biochemical analyses. Serum glucose levels and complete lipid profile (triglycerides, total cholesterol and HDL-cholesterol) were determined using an auto analyzer (Konelab, Finland). LDL-cholesterol was calculated using the Friedewald formula. Serum concentrations of insulin, leptin and adiponectin were measured using rat specific enzyme immunoassays (Alpco Diagnostics, USA). A homeostasis model assessment of insulin resistance (HOMA-IR) was carried out to evaluate fasting insulin resistance using the formula: glucose (mmol l^{-1}) × insulin (μIU ml^{-1})/22.5.

Statistical analysis

Data were analysed using Statistical Package SPSS for Windows version 11.5. Variables were presented as mean ± standard error. Variables exhibiting non-Gaussian distribution were logarithmically transformed and adiponectin was power transformed with lambda value 0.55. Lambda value was generated from MedCalc software version 11.1.0. Analysis of variance (ANOVA) and post-hoc tests were done for the comparisons among groups.

RESULTS

Biochemical parameters and body weight

Control rats continued to gain weight throughout the study, while after 12 and 16 weeks of incense exposure, a significant reduction in rat weight was observed in the incense exposed group compared to controls (Figure 1). The correlation between incense and leptin was shown in Figure 2, weight was inversely associated with serum leptin levels ($r = -0.209$, $p = 0.035$). Table 1 shows the biochemical parameters in rats exposed to incense smoke for 16 weeks. The results for glucose, insulin and HOMA-IR showed no significant differences between incense exposed rats compared to controls at the corresponding time points. However, after 4, 8, 12 and 16 weeks of exposure, the

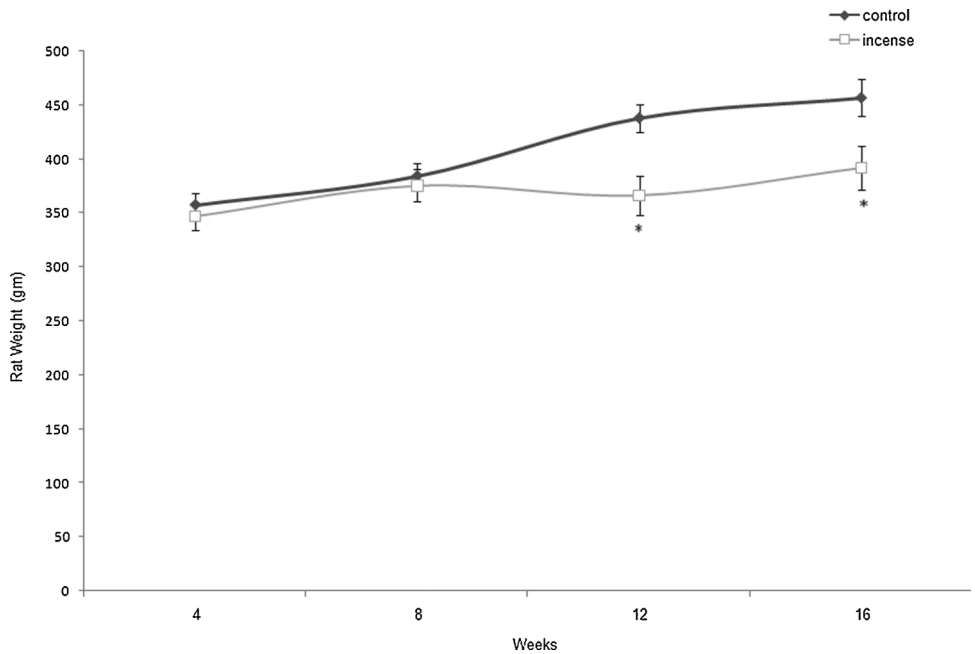

Figure 1. Body weight of rats exposed to incense for 16 weeks. Values represent the mean ± standard error. *p* values indicate statistical significance compared to controls at the same time points: $^{*}p < 0.05$

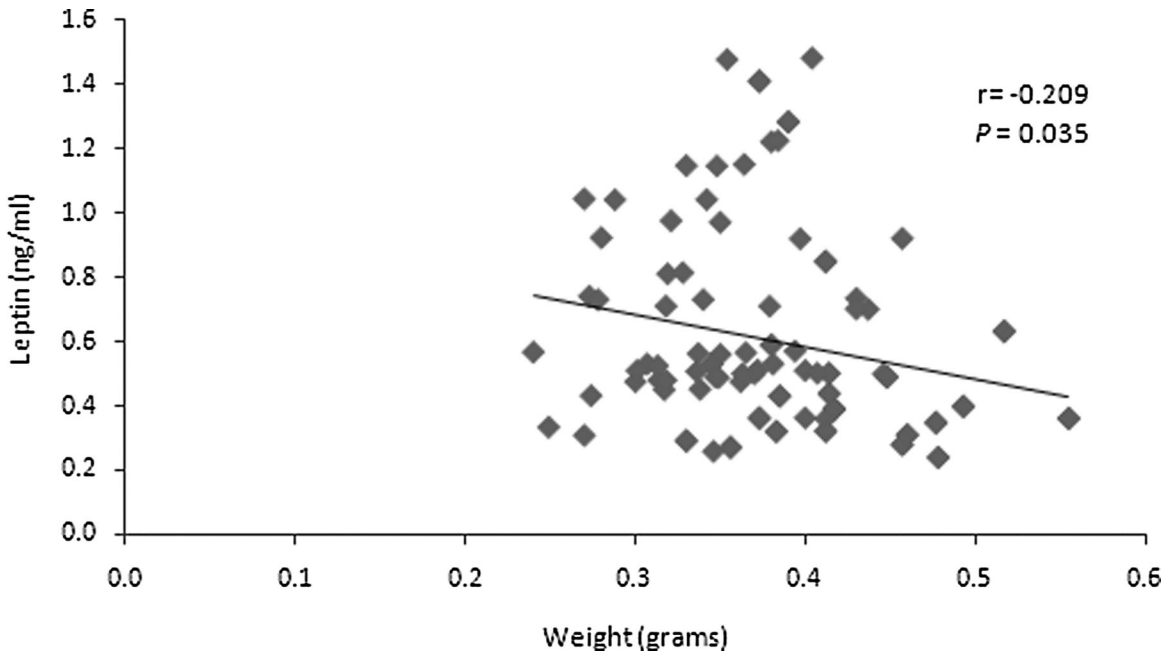

Figure 2. Correlation between leptin and body weight in rats exposed to incense for 16 weeks

triglyceride levels were significantly increased by 22.2%, 31.8%, 45% and 69%, respectively ($p < 0.05$), compared to controls at the corresponding time points. HDL-cholesterol levels were significantly decreased by 16.2% and 19.4% ($p < 0.05$), after 12 and 16 weeks of exposure, respectively. After 4 and 8 weeks of exposure, LDL-cholesterol levels were significantly decreased by 29% and 40%, respectively ($p < 0.05$), compared to controls at corresponding time points. However, after 12 and 16 weeks of exposure, the differences in LDL-cholesterol between incense and control levels were no longer significant, indicating that the incense effect on this parameter was transient. Cholesterol levels were not different between the incense-exposed and control groups at any time point.

Adipocytokines

After 4 and 8 weeks of exposure, leptin levels were significantly increased by 83% and 75%, respectively ($p < 0.05$), compared to controls at corresponding time points (Figure 3). However, after 12 and 16 weeks of exposure, leptin levels were reduced and the differences in leptin levels were no longer significant, indicating that the incense effect on this parameter was transient. Figure 4 shows adiponectin levels in rats exposed to incense smoke for 16 weeks. There were no significant changes in adiponectin levels between incense-exposed and control group at any time point.

DISCUSSION

The widespread use of incense in the Arabian tradition for several domestic purposes has prompted the need for investigation on whether incense smoke exposure might carry hazards for human health. There is little information on the potential relations between the Arabian incense and human health.[17] It has been reported that exposure to incense smoke may be associated with many adverse health effects,

Table 1. Biochemical parameters in rats exposed to incense for 16 weeks ($n = 12$)

	4 weeks		8 weeks		12 weeks		16 weeks	
	Control	Incense	Control	Incense	Control	Incense	Control	Incense
Glucose ($mmol\,l^{-1}$)	5.5 ± 0.26	5.6 ± 0.26	4.19 ± 0.5	4.4 ± 0.29	5.13 ± 0.4	5.01 ± 0.2	5.18 ± 0.6	4.8 ± 0.4
Insulin ($\mu IU\,ml^{-1}$)	5.64 ± 0.63	4.34 ± 0.54	7.02 ± 0.7	7.5 ± 0.7	5.8 ± 0.8	4.4 ± 0.5	3.0 ± 0.4	2.8 ± 0.5
HOMA-IR	0.73 ± 0.01	1.25 ± 0.04	1.84 ± 0.3	1.87 ± 0.06	2.04 ± 0.4	1.25 ± 0.05	0.62 ± 0.02	0.50 ± 0.01
Triglyceride ($mmol\,l^{-1}$)	0.90 ± 0.04	$1.1 \pm 0.04^{*}$	0.91 ± 0.04	$1.2 \pm 0.04^{*}$	1.0 ± 0.04	$1.45 \pm 0.09^{*}$	0.8 ± 0.04	$1.49 \pm 0.13^{*}$
Cholesterol ($mmol\,l^{-1}$)	1.72 ± 0.05	1.6 ± 0.04	1.7 ± 0.05	1.5 ± 0.03	1.67 ± 0.05	1.83 ± 0.1	1.6 ± 0.05	1.5 ± 0.04
HDL ($mmol\,l^{-1}$)	0.67 ± 0.02	0.62 ± 0.02	0.71 ± 0.03	0.63 ± 0.03	0.68 ± 0.02	$0.57 \pm 0.03^{*}$	0.67 ± 0.02	$0.54 \pm 0.03^{*}$
LDL ($mmol\,l^{-1}$)	0.61 ± 0.02	$0.43 \pm 0.02^{*}$	0.55 ± 0.01	$0.33 \pm 0.02^{*}$	0.51 ± 0.01	0.6 ± 0.09	0.5 ± 0.01	0.42 ± 0.01

Values represent means $\pm$ standard error. p values indicate statistical significance compared to controls at the same time points: $^{*}p < 0.05$.

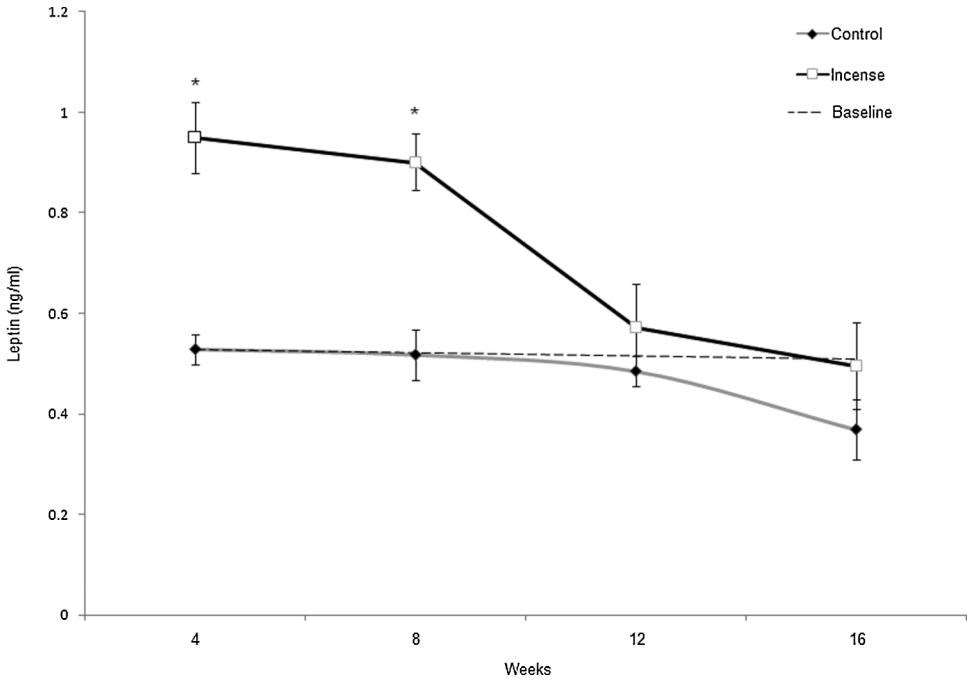

Figure 3. Leptin levels in rats exposed to incense for 16 weeks. Values represent the mean ± standard error. *p* values indicate statistical significance compared to controls at the same time points: $^{*}p < 0.05$

including, cancer.[4] It has also been shown that Asian temple workers, who are exposed to incense, had significantly higher levels of DNA damage and significant reduction of DNA repair capacity than control workers.[18]

The current study investigated, for the first time, the effect of chronic incense exposure on the regulation of body weight, lipid profile and adipocytokines in rats. Like any cigarette smoke and wood smoke, incense smoke contains

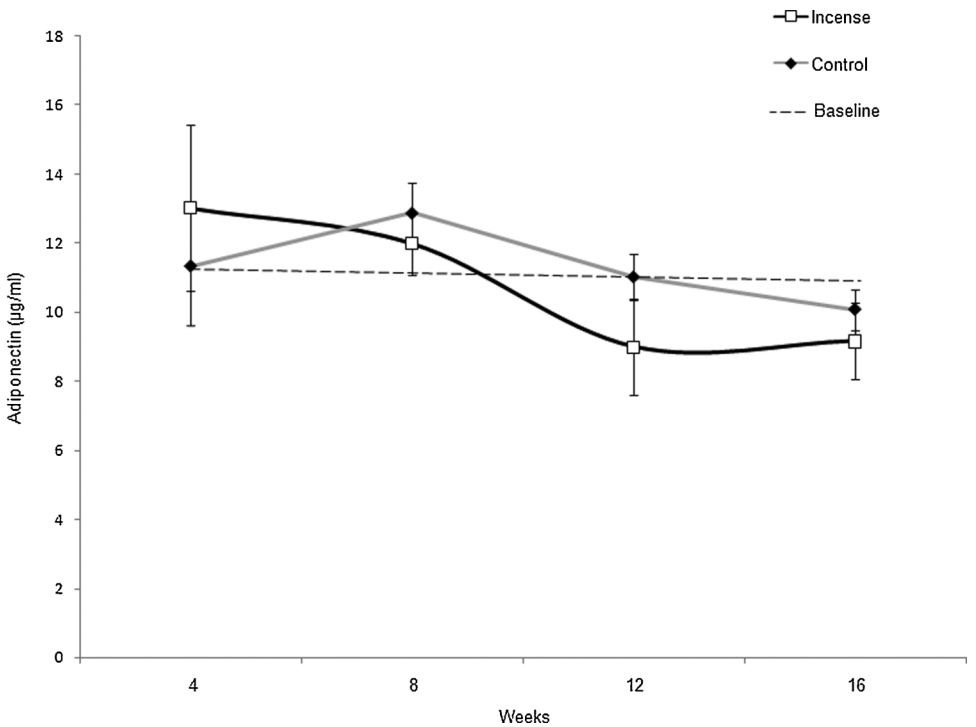

Figure 4. Adiponectin levels in rats exposed to incense for 16 weeks. Values represent the mean ± standard error

particulate matter, gas products (carbon monoxide, carbon dioxide, nitrogen dioxide and sulfur dioxide) and other organic compounds (benzene, toluene, xylenes, aldehydes and polycyclic aromatic hydrocarbons), which have been shown to harm human health.[4] The combustion of incense, wood, cigarette and the burnining of candles are an important source of residential indoor particulate matter, especially in the 2.5 μm size range and below.[19–25] Interestingly, incense burning produces over 4 times more particulate matter than cigarette smoke.[26] Incense smoke condensates had genotoxic effects, such as induction of sister chromatid exchange, and the genotoxicity of certain incense smoke condensates in mammalian cells were higher than those of tobacco smoke condensates.[27]

Our data showed that incense smoke exposure was associated with an altered lipid profile, inducing an increment in circulating triglycerides and a reduction in HDL-cholesterol concentrations. Consistently, exposure to cigarette smoke lowers HDL and elevates triglyceride concentrations.[28] Our results also showed that exposure to incense for 4 and 8 weeks was associated with transiently increased leptin levels. In contrast, reports indicate that cigarette smokers reduced leptin levels in humans[29,30] and mice.[31]

Exposure to incense smoke for 12 and 16 weeks was associated with a decrease in body weight, in contrast with the reduction in leptin levels in the incense-exposed group after 12 and 16 weeks compared with the 4 and 8 weeks of exposure. Cigarette smoking has been associated with decreased adiponectin levels in humans[32] and mice.[33] In contrast, our data showed no effect of incense exposure on adiponectin levels. Glucose, insulin and HOMA-IR were not significantly different between incense-exposed and control group indicating no effect of incense on glucose metabolism.

CONCLUSION

This study demonstrates that incense smoke alters body growth, the lipid profile, and transiently, the leptin levels in Wistar albino rats. These effects of incense smoke on health and the mechanisms behind them need to be further studied.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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