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Original Article

Weight Loss Associated with Reduced Visceral Fat Deposition Mediated via Tea-induced Alterations in Adiponectin and Insulin Activities

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ABSTRACT

Background and Aim: Weight decreasing potential of Lipton tea (product of *Camellia sinensis*) was elucidated considering its impact on adiponectin and insulin concentration, which in turn influences the activities of adiponectin and insulin on their target cells. Hence this study was conducted forming tea nutritional basis for body weight and obesity control. **Materials and Methods:** Adiponectin, insulin, insulin resistance, free fatty acids, visceral fat depositions, body weight of male Wistar rats orally administered 200mg/kg, 400mg/kg, and 800mg/kg of Lipton tea as well as 500 mg/kg of metformin (a potent drug for weight loss) in various groups were measured to ascertain certain weight decreasing mechanism of Lipton tea. **Results:** Following the administration of Lipton tea for 28 days, it increased adiponectin and decreased insulin and insulin resistance, free fatty acids, visceral fats and body weight in a dose dependent manner when compared to control. **Conclusion:** Weight decreasing potential of Lipton tea via reduction in visceral fats is associated with increased adiponectin as well as decreased insulin concentration and decreased insulin resistance induced by the tea.
Key words: Adiponectin, *Camellia sinensis,* Insulin, Tea, Visceral fat deposition, Weight loss.

INTRODUCTION

Obesity (severe overweight) has been associated to increase in body weight.[1-2] Studies have linked changes in body weight to nutritional implications and pathophysiological conditions.[3-5] Storage of fats in adipose tissues and glucose metabolism plays role in building the body calorie which in turns contributes to body weight.[6-7] Overweight and obesity has been suggested to be associated with some conditions like hypertension, arteriosclerosis, diabetes mellitus, arthritis etc.[8-10] Thus, control of body weight became a concern as it can aid body wellness and fitness. Exercise and dietary measures has been employed as reports suggest that they play vital role in regulation of body weight.[11-12]

Lipton tea (a product of *Camellia sinensis*) has been used in folk and traditional medicine for body weight regulation and previous studies has shown that Lipton tea extract causes reduction in body weight both in animals and humans,[13-14] but the underlying mechanism through which Lipton tea affects body weight is not fully explained. In this study the weight decreasing potential of Lipton tea was elucidated by investigating its impact on adiponectin and insulin concentration, which in turn influences the activities of adiponectin and insulin on their target cells. It was hypothesized that tea nutritional basis for body weight and obesity control is dependent on its impact on adiponectin and insulin which has been reported to play essential role in body weight; this impact could be reflected in visceral fat depositions and free fatty acids, thus these variables were also measured since their concentration in the body is directly proportional to body weight.[15-18] Hence, this study developed relevant support for the recommendation of Lipton tea as nutrient for body weight control.

MATERIALS AND METHODS

Subjects

Twenty-five male Wistar rats weighing 150 – 200g were bought from Animal House Units, Gregory University Uturu, Abia State. The rats were acclimatized for two weeks and were fed with pelleted grower's marsh throughout the duration of the experiment. Humane care and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the Ethics Review Committee of the institution for the purpose of this study.

Procurement and Preparation of Lipton Tea

Lipton yellow label tea (Unilever PLC, Nigeria) was purchased from Ukwunwangwu market in Uturu, isiukwuato LGA of Abia State. Lipton tea was mixed with 100ml of distilled water in a beaker. The mixture was allowed to boil for 20min. The Lipton

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tea extract obtained from boiling the mixture was evaporated to make it dry in a rotary evaporator (Stuart, model RE-300, UK). Concentration was determined: Extract concentration (mg/ml) = (W2-W1)/100, where W1 (mg) = Weight of beaker and W2 (mg) = Weight of beaker + 100 ml of extract evaporated to dryness.

Administration Protocol

Animals were grouped into 5 groups (n=5). All administrations were given orally on daily basis for 28 days. Metformin dose was according to Molepo M, *et al.*[19] and Lipton tea dose was according to Anorue EC.[13]

Group 1: (control group): Received distilled water

Group 2: Received 500 mg/kg of metformin

Group 3: Received 200mg/kg of Lipton tea

Group 4: Received 400mg/kg of Lipton tea

Group 5: Received 800mg/kg of Lipton tea

Sample Collection

Samples were collected from animals on anesthesia (sodium pentobarbital 65mg/kg i.p). Blood sample was collected from animal in each group via cardiac puncture in plain and heparinized sample bottles. Visceral fat and other internal organs were also harvested and all samples collected were used for laboratory assay.

Assay for Adiponectin

Lysates from the visceral fat depot were used to perform the assay. Adiponectin were determined using their specific Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology, China). The protocol of the kit was strictly adhered as stated in the manufacturer's manual. The concentrations of the adiponectin were expressed in ng per 100 mg of tissue or in ng/ml.

Assay for Insulin and Insulin Resistance

Fasting serum insulin levels were measured using ELISA kit from EMD Millipore (Billerica, MA, USA). The Homeostasis Model Assessment of Insulin Resistance (HOMA1-IR) index was calculated using fasting plasma insulin and glucose by the following formula:

HOMA1-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5.

Assay for Free Fatty Acid

A known amount of each harvested fat pad (subcutaneous and visceral) of adipose tissue was placed in Dulbecco's Modified Eagle Medium (DMEM) with antibiotics (50mg.ml-1 gentamicin) or in KBEBS-Ringer's solution (pH = 7.4). After overnight incubation at 37°C, 5% CO_2; samples were centrifuged and supernatants frozen at -80°C. FFA were analysed with a commercial kit (Wako Chemicals, Richmond, USA), in accordance to the manufacturer's instructions.

Measurement of Visceral Fat Depositions and Body Weight

Electronic top loading balance (Mettler Toledo Series) to the nearest 0.01 g was used to measure visceral fat depositions and body weight. The absolute weights of epididymal fat, mesenteric fat and retroperitoneal fat depots were measured as intraperitoneal visceral fat.

Statistical Analysis of Data

The data obtained from the laboratory assays were statistically analyzed using GraphPad Prism (version 8). One-way analysis of variance (ANOVA) was used to determine statistical significance at P<0.05. Multiple comparisons were done between all groups. Results were expressed as mean ± SEM.

RESULTS

Tea-induced Alterations in Adiponectin, Insulin, Insulin Resistance and Free Fatty Acids

Figure 1 showed that Lipton tea induced significant dose dependent increase in adiponectin. Reduction in insulin and insulin resistance induced by Liption tea was observed (Figure 2) and significant dose dependent decrease in free fatty acids mediated by Lipton tea was also observed (Figure 3).

Tea-induced Alterations in Visceral Fat Deposition and Body Weight

Weight loss potential of Lipton tea was determined by considering the impact of Lipton tea on visceral fat depositions and body weight, which are measures for body mass composition. Lipton tea showed significant dose dependent decrease in grams of visceral fat depositions and body weight (Table 1).

DISCUSSION

The weight decreasing potential of Lipton tea and certain underlined associated physiological variables were investigated in order to elucidate the physiological basis linked with the use of Lipton tea for body weight control. In this study we measured the aspect of adiponectin and insulin activities; it was observed that Lipton tea increased adiponectin and

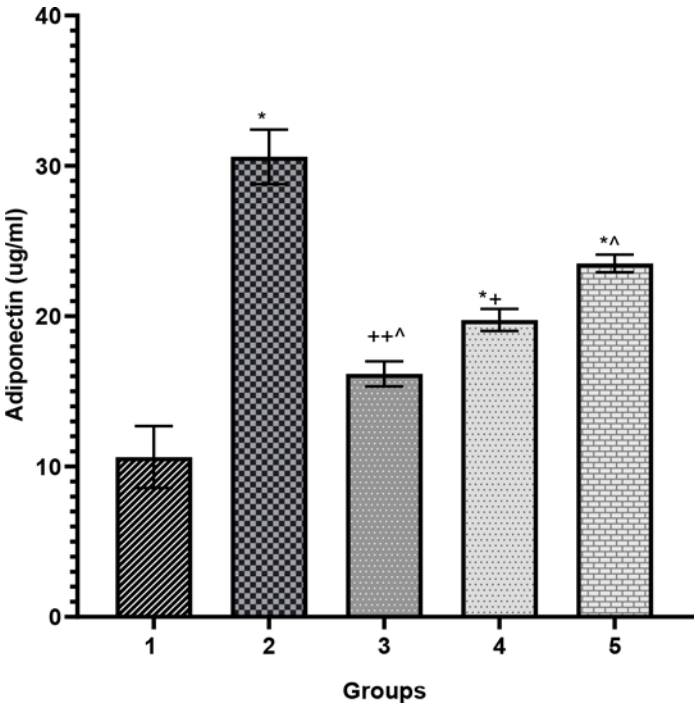

Figure 1: Adiponectin levels (ug/ml).
Values are expressed as mean ± SEM.
* indicate values that are significantly different from control.
+ indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01).
^ indicates values that are significant between Group 3, 4 and 5 (P<0.01).
Group 1= control (standard diet and water); Group 2 = 500 mg/kg of metformin; Group 3, 4 and 5 = 200mg/kg, 400mg/kg, 800mg/kg of Lipton tea respectively.

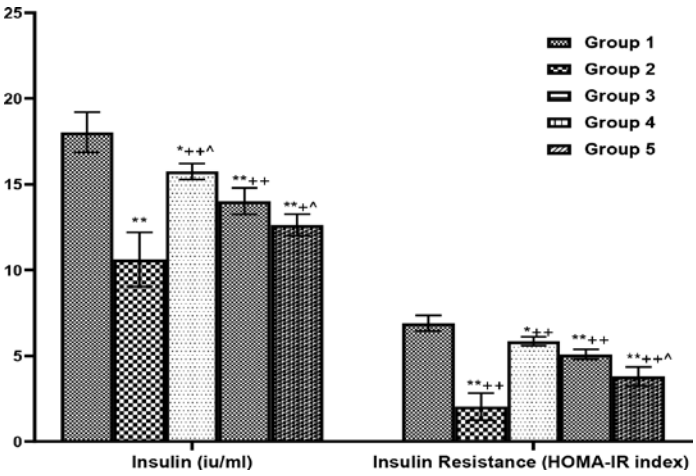

Figure 2: Insulin and Insulin Resistance.
Values are mean ± SEM.
* indicate values that are significantly different from control (**P < 0.01).
+ indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01).
^ indicates values that are significant between Group 3, 4 and 5 (P<0.01).
Group 1= control (Standard diet and water); Group 2 = 500 mg/kg of metformin; Group 3, 4 and 5 = 200mg/kg, 400mg/kg, 800mg/kg of Lipton tea respectively.

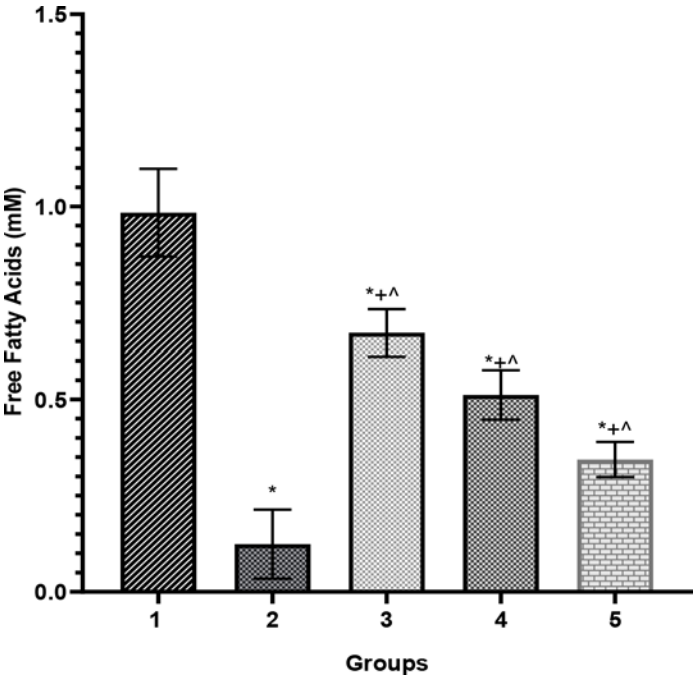

Figure 3: Free fatty acids (mM).
Values are mean ± SEM.
* indicate values that are significantly different from control (**P < 0.01).
+ indicates values that are significantly different from animals in Group 2 (+P < 0.05) (++ P < 0.01).
^ indicates values that are significant between Group 3, 4 and 5 (P<0.01).
Group 1= control (Standard diet and water); Group 2 = 500 mg/kg of metformin; Group 3, 4 and 5 = 200mg/kg, 400mg/kg, 800mg/kg of Lipton tea respectively.

Table 1: Visceral fat mass and body weight in all groups.

Groups	Visceral fat mass (g)	Body weight (g)
Group 1	8.480 ± 1.428	187.6 ± 17.27
Group 2	0.848 ± 0.418**	103.2 ± 6.301**
Group 3	6.478 ± 0.983*$^{++}$	149.8 ± 7.629**$^{++}$
Group 4	3.046 ± 0.639**$^{++}$^^	130.0 ± 5.431**$^{++}$^
Group 5	1.470 ± 0.249**^^	110.8 ± 8.379**^^

Values are mean ± SEM. * indicate values that are significantly different from control group (*P < 0.05, **P<0.01). + indicates values that are significantly different from animals in Group 2 ($^{+}$P < 0.05, $^{++}$P<0.01). ^ indicates values that are significant between Group 3, 4 and 5 (^P < 0.05, ^^P<0.01)

this can account for decrease in body weight of animals and humans since increased adiponectin concentration has been reported to decrease body fat. Thus serum levels of adiponectin decrease with increased body weight and are positively associated with insulin sensitivity.[20-21]

Obesity and overweight has been reported to result from increased insulin and insulin resistance; since insulin increases the storage of sugars and free fatty acids in the adipose tissues. A higher level of insulin also prevents fat from being broken down for energy. However, chronic high levels of insulin, also known as hyperinsulinemia, can lead to excessive weight gain.[22-24] In this study, Lipton tea showed potency in decreasing insulin and insulin resistance. This suggested a potent inhibiting role of the tea on insulin thereby reducing its activities which in turn causes decrease in body weight.

Free fatty acid levels are increased in obesity; they cause insulin resistance in all major insulin target organs (skeletal muscle, liver, endothelial cells) and have emerged as a major link to obesity.[25] This is because the enlarged adipose tissue mass releases more free fatty acids and its clearance may be reduced.[25-26] The evaluated impact of Lipton tea on free fatty acid, visceral fats and body weight showed significant decrease. This is an indication of the efficacy of Lipton tea to cause reduction in body weight.

Visceral Fat Deposition (visceral fat depot) is one of the first depots to be developed which function for protection of the internal organs and also provides a reserve source for energy if needed by the animal.[27] Abnormally high deposition of visceral adipose tissue is known as visceral obesity, thus contributes to body weight.[28]

Metformin a potent drug for weight loss including diabetes and obesity[19] was compared to the weight loss potential of Lipton tea. The study results suggest that Lipton tea followed the potent trend of metformin in reducing body weight via reduction in adiponectin, insulin and other parameters.

CONCLUSION

Weight decreasing potential of Lipton tea via reduction in visceral fats is associated with increased adiponectin as well as decreased insulin concentration and decreased insulin resistance induced by the tea. Thus Lipton tea can be recommended as weight regulatory diet.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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