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Polar *Curcuma longa* extract inhibits leptin release by adipose tissue derived from overweight and obese people

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Abstract

Obesity escalates at an alarming rate worldwide. It is associated with chronic low-grade inflammation, which is implicated in the pathogenesis of type 2 diabetes and cardiovascular diseases. Adipose tissue is a primary site of obesity-induced pro-inflammatory factors such as leptin. Curcuminoids are potent anti-inflammatory agents; however, evidence on the polar fraction of *Curcuma longa* is scarce and the effect on leptin release has never been investigated. Therefore, we will investigate the influence of aqueous *C. longa* extract on leptin release from human subcutaneous adipose tissue (SAT). To achieve this, SAT explants were obtained from patients who underwent abdominal surgery. Patients were both males (67%) and females (33%). Their average body mass index (BMI) and age were 33.4 kg/m^2 and 43 years, respectively. Tissue explants were treated in triplicate with or without 0.1 and 1 mg/mL aqueous *C. longa* extracts and incubated for 24 and 48 h. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of leptin secreted by SAT. We have shown that 24 h treatment of SAT with 0.1 and 1 mg/mL of *C. longa* polar extract inhibited the release of leptin, while 48 h treatment of SAT with only 1 mg/mL of *C. longa* polar extract inhibited leptin release. Concentration of basal leptin released from SAT derived from females was higher than from males. However, this could be because females were morbidly obese. Our data demonstrate for the first time the inhibitory effect of aqueous *C. longa* extract on leptin release, shedding light on the role of leptin and adipose tissue in the mechanism of *C. longa* in reducing low-grade inflammation. This avoids the poor solubility and consequently the low bioavailability disadvantages of curcuminoids.

Keywords

adipose tissue, *Curcuma longa*, inflammation, leptin

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Introduction

Obesity escalates at an alarming rate worldwide. In 2014, World Health Organization[1] reported that more than 600 million were obese (~13%) worldwide. In Palestine, the prevalence has been shown to be approximately four times among women (49%) and two times among men (30%) higher than the global prevalence mentioned above.[2]

Obesity has been shown to be responsible for an estimated 216,000 deaths accounting for about 1 in 10 deaths in US adults.[3] This is because obesity is

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Table 1. Criteria of the patients.

Patient	Age	Gender	BMI	Reasons for admission	Past surgical history	Chronic disease/medication
S1	46	Female	35	Appendectomy	None	None/none
S2	38	Female	41	Appendectomy	Cesarean section	None/none
S3	36	Male	28	Appendectomy	None	None/none
S4	57	Male	31	Hernia repair	Left-side varicocelloctomy and hernia repair	Mild hypertension/none
S5	33	Male	32	Appendectomy	None	None/none

BMI: body mass index.

raising the risk of chronic diseases such as cardiovascular diseases, type 2 diabetes, and some cancers.[4] The etiology of obesity and its related chronic diseases is complex and includes genetics and environmental factors. However, accumulating evidence suggests that chronic low-grade inflammation is implicated in the pathogenesis of obesity-associated chronic diseases.[5] A primary source of low-grade inflammation observed in obese patients is adipose tissue. Therefore, compounds that attenuate the inflammatory response associated with obesity may prove useful in the medical management of patients with type 2 diabetes and cardiovascular diseases.

Nowadays, phytochemicals are proved to be a potent diet with the potential to diminish inflammation, among these is *Curcuma longa*.[6] Curcuminoids (mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin) are considered as key active constituents of *C. longa* and are reported to be potent anti-inflammatory agents.[6] Very recently, in a clinical trial, curcuminoids have been shown to either decrease or to have no influence on leptin release, which is a pro-inflammatory factor released from adipose tissue.[7,8] However, curcuminoids are present in the organic extract and the evidence of aqueous extract effect on inflammation is scarce and the effect on leptin level has never been investigated. Therefore, in this study, we aim to investigate, for the first time, the influence of polar *C. longa* extract on the release of leptin from human abdominal subcutaneous adipose tissue (SAT).

Materials and methods

Materials and reagents

Gentamicin, glucose, and bovine serum albumin (BSA) were purchased from Sigma (USA) and M199 media from Invitrogen (USA). All chemicals were purchased from Riedel-de Haen (Germany). Sterile mesh gauze was purchased from NISSAN Medical industries (Israel). Leptin ELISA kit was purchased from R&D systems (USA).

C. longa *extraction*

In total, 25 g of *C. longa* was weighed and exhaustively extracted by adding 100 mL of *n*-hexane and 150 mL of 50% ethanol in distilled water. The mixture was placed in a shaker for 72 h at 25°C with continuous shaking at 200 r/min. Then, it was filtered by Whitman's No. 1 filter paper using suction flask and Buchner funnel. The resulting liquid filtrate was separated by a separatory funnel into two phases: the lower aqueous phase and the upper organic phase. Then, aqueous extract was placed in a rotary evaporator for 1 h at 40°C to evaporate any leftover organic solvents. To get rid of the remaining water, aqueous extract was dried using freeze dryer.[9] Finally, aqueous *C. longa* extract was dissolved in M199 media.

Abdominal SAT sample collection and culture

Human abdominal SAT explants were obtained from five patients, males and females, who underwent surgery for reasons described in Table 1. Adipose tissue culture was performed as described previously[10] with slight modifications. After the last washing step, tissue explants were incubated for 24 and 48 h with or without 0.1 and 1 mg/mL aqueous *C. longa* extract. Subsequently, the media was stored in a freezer at −20°C for ELISA test. The study was approved by the local medical ethical committee (IRB committee of An-Najah National University, Nablus, Palestine). All patients gave informed, signed consent to participate in the study.

Leptin quantification by ELISA

Secreted leptin was measured in culture media by DuoSet ELISA kit according to the manufacturer's instructions (R&D Systems, USA).

Statistics

Statistical analysis was performed employing GraphPad Prism software. Comparison between two groups was performed by nonparametric Mann–Whitney test, while comparison between three groups was analyzed via one-way analysis of variance (ANOVA) followed by Dunn's post hoc test. For correlation results, spearman correlation test was used. Results were considered to be statistically significant at $P<0.05$.

Results

Criteria of patients

The number of patients included in our study was five. They were both males (67%) and females (33%) with a body mass index (BMI) ranging from 28 to 41 (mean=33.4) kg/m^2 who underwent surgical operations for few reasons such as appendectomy and hernia repair as mentioned in Table 1. The patients were free of medication for chronic diseases; however, prior to operation, they were given antibiotics. Most of the patients had no past surgical history except S2 and S4, who had cesarean section and left-side varicocelloctomy and hernia repair surgeries. The average age of the patients was approximately 43 years old.

Aqueous C. longa *extract effect on leptin secretion*

Abdominal SAT explants were incubated in triplicate for 24 and 48 h with or without aqueous *C. longa* extracts (0.1 and 1 mg/mL**)**. Leptin quantity released into media was quantified employing ELISA technique. As shown in Figure 1(a), 0.1 and 1 mg/mL of *C. longa* extracts significantly reduced the release of leptin from SAT by approximately 50%. After 48 h, it appeared that 1 mg/mL *C. longa* extract treatment resulted in significant decrease of leptin release by approximately 30% (Figure 1(b)).

Effect of gender and BMI on basal leptin quantity released by SAT

As demonstrated in Figure 2(a), the correlation coefficient between leptin concentration and BMI

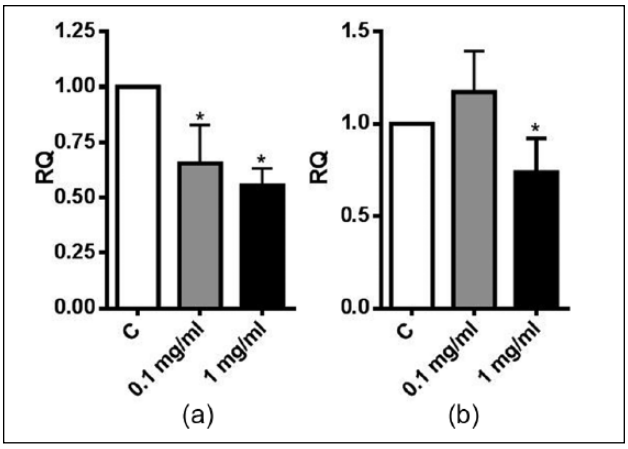

Figure 1. Aqueous *Curcuma longa* extract inhibits leptin secretion. SAT explants of each patient were incubated with or without 0.1 and 1 mg/mL of aqueous extract for (a) 24 and (b) 48 h. Secreted quantities of leptin in the media were determined by ELISA. Results were depicted as relative quantities (RQ) compared to the control (without extract; C). *$P<0.05$ versus control. Error bars, SEM.

was high (R=0.6). To further understand this, we divided the patients into two groups according to their BMI, that is, patients with BMI<35 and patients with BMI≥35. It appeared that basal leptin concentration released from SAT derived from patient with BMI<35 was fivefold lower than leptin released from SAT derived from patient with BMI≥35 (Figure 2(b)). Furthermore, SAT explants derived from females released more leptin than SAT explants derived from males (Figure 2(c)). Both BMI and gender significantly ($P<0.05$) influenced leptin quantity released from SAT. However, due to the level of high multicollinearity, multivariable analysis was not feasible.

Effect of gender and BMI on leptin response to the treatment

As demonstrated by Figure 3(a), the correlation coefficient between leptin response and BMI was 0.4. To further understand this, we divided patients into two groups according to their BMI, that is, patients with BMI<35 and patients with BMI≥35. It appeared that leptin response in SAT derived from patients with BMI<35 was decreased twofold, while leptin response in SAT derived from patient with BMI≥35 was not influenced (Figure 3(b)). SAT explants derived from males responded significantly to polar *C. longa* extract treatment, while SAT explants derived from females did not respond (Figure 3(c)). BMI and gender significantly ($P<0.05$) influenced leptin response to *C.*

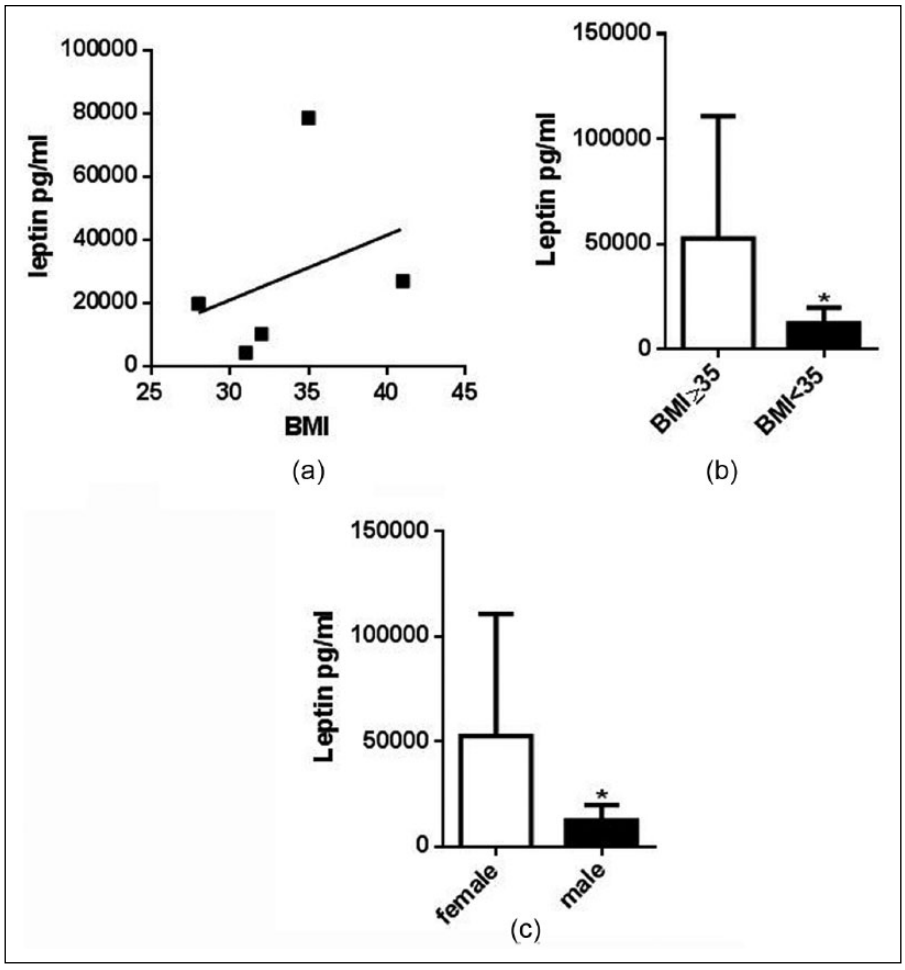

Figure 2. BMI and gender effect on basal leptin secretion. (a) Correlation between BMI and leptin released quantity was determined employing Spearman correlation test (R = 0.6). (b) BMI effect by dividing patients into two groups according to their BMI. (c) Gender effect. SAT explants of each patient were incubated for 48 h without treatment. Secreted basal quantities of leptin in the media were determined by ELISA. *$P < 0.05$ versus BMI ≥ 35 or females. Error bars, SEM.

longa extract treatment. However, due to the level of high multicollinearity, multivariable analysis was not feasible.

Discussion

Adipose tissue is a primary source of obesity-induced pro-inflammatory factors, including leptin hormone. Leptin is highly investigated due to its circulating level that is proportional to fat mass and reflects inflammation grade.[11] Therefore, we investigated the effect of polar *C. longa* extract on human adipose tissue and it appeared to downregulate leptin release. These results are comparable to curcuminoid observed anti-inflammatory properties, including the inhibition of leptin.[6] However, our results demonstrate the effect of the polar soluble extract of *C. longa* rather than curcuminoids, which has never been demonstrated earlier in human or animal studies. Only very few studies focused on the role of the aqueous extract, and very recently, a polysaccharide fraction of the polar extract has been shown to inhibit inflammation.[12] Furthermore, *C. longa* extract has been shown to induce lipolysis and consequently to reduce leptin release in vitro and in vivo.[13] As leptin level is a predictor of the total mass of fat in the body, we could assume that our polar extract inhibits lipid accumulation and therefore leptin release. This, on long term, will reduce leptin resistance and related chronic diseases. This is strengthened by a recent study, where polar extract has been shown to have anti-diabetic properties.[14]

In an earlier study, it was shown that leptin quantity released from adipose tissue derived from females was approximately twofold higher than

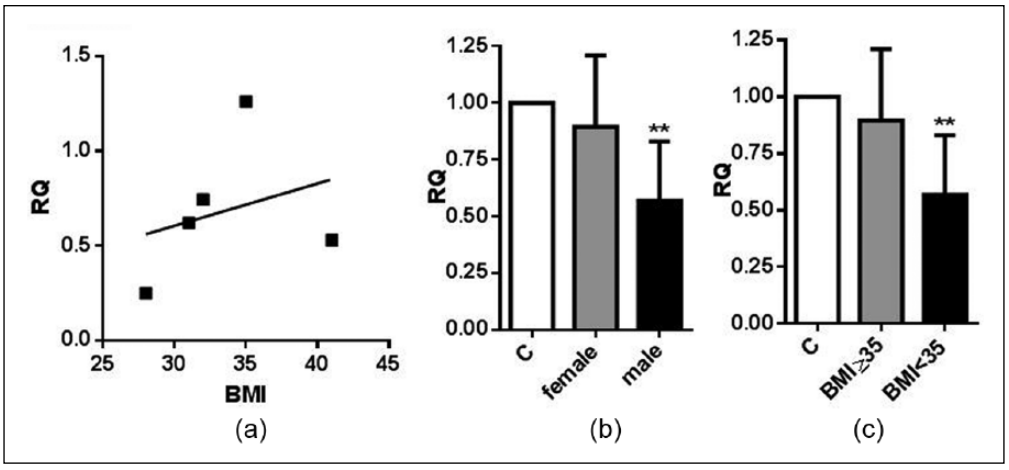

Figure 3. BMI and gender effect on leptin response. (a) Correlation between BMI and leptin response was determined employing Spearman correlation test (R = 0.4). (b) BMI effect by dividing patients into two groups according to their BMI. (c) Gender effect. SAT explants of each patient were incubated for 48 h with or without 1 mg/mL of aqueous extract. Secreted quantities of leptin in the media were determined by ELISA. Results were depicted as relative quantities (RQ) compared to the control (without extract; C). *$P < 0.05$ versus BMI ≥ 35 or females. Error bars, SEM.

from males.[15] This is in agreement with our findings. Interestingly, the females in our study were morbidly obese, while males were not. Therefore, this could be due to BMI difference. In addition, tissues obtained from females did not respond to the treatment, while tissues obtained from males did, again this could be due to BMI difference. However, due to high level of multicollinearity, multivariable analysis was not feasible.

In conclusion, *C. longa* aqueous extract inhibits leptin release, revealing a promising anti-inflammatory property. This suggests that it has a potential as a herb to prevent or manage obesity and its related chronic disorders. Our study is very important because using polar soluble extract will overcome poor solubility and consequently poor bioavailability of curcuminoids, which has hindered its use so far. However, this needs to be further investigated in the future.

Declaration of conflicting interests

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