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The Effect of Omentin on the Contractility of Rat Uterus

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Abstract

Adipokines are a group of proteins that are synthesized from adipose tissue. Omentin is a type of adipokine which is detected in human serum and it is evaluated as a secreted factor from adipose tissue, placenta and ovarium. The knowledge about the effect of omentin on reproduction is limited. In the presented study, investigation of omentin effect on *in vitro* uterine contractions was evaluated. The uteruses of the rats which were collected from 20 virgin female rats, were isolated and suspended in the isolated organ bath. The experimental protocols were carried out in 3 groups. The effect of omentin on spontaneous uterine contractions in protocol 1, induced by oxytocin (2.5 mIU/mL) in protocol 2, and induced uterine contractions with $PGF_2\alpha$ (10^{-6} M) in protocol 3 was tested. The effect of omentin on isolated uterine tissues was tested with the administration of 10, 100 and 300 ng/mL omentin. 300 µL of distilled water was applied to the control of each group. In this study, 10, 100 and 300 ng/mL of omentin applied for 10 minutes did not have an effect on uterine contractility in all study groups ($p>0.05$). As a result, further studies with higher doses and longer incubation times are recommended.

Keywords: Adipokines, in vitro, omentin, uterus, smooth muscle, rat.

INTRODUCTION

Adipokines play an important role in many biological events, including immune function, inflammation, hemostasis, vascular biology, hematopoiesis, cell proliferation, angiogenesis, regulation of energy metabolism, and blood pressure. Although not yet routinely used, adipokines have also been found to be involved in the control and regulation of reproduction (Radin et al. 2009; Aktaş et al. 2013; Reverchon et al. 2014).

Omentin is an adipokine consisting of 313 amino acids and expressed from visceral adipose stromal vascular cells rather than subcutaneous adipose tissue. Omentin is a fat hormone not only detected in adipose tissue but also detected in the placenta and ovary. It has recently been determined that omentin plays an anti-inflammatory role in vascular smooth muscle cells. Plasma levels and gene expression of omentin are lower in obesity, impaired glucose tolerance, and type 2 diabetes mellitus. Omentin studies on reproduction are limited and it is thought to be associated with polycystic ovary syndrome (Yang et al. 2006; Comninos et al. 2014).

Yamawaki et al. (2010) investigated the effect of omentin on vascular tissues and showed that tissues incubated with 300 ng/mL omentin for 30 minutes inhibited noradrenaline contractions and this relaxation was inhibited by a nitric oxide synthetase inhibitor. Yamawaki et al. (2010) reported that omentin has a relaxing effect on isolated blood vessels and this effect is regulated by endothelial-derived nitric oxide (NO). Mustafa et al. (2020) compared obese pregnant human myometrial contractions with normal pregnant human myometrial contractions and found that omentin reduced spontaneous contractions in obese pregnant human myometrium. This reduction was not observed when the tissue was treated with oxytocin. Mustafa et al. (2021) tried 1nmol/L -10 µmol/L omentin in healthy pregnant human myometrium and found that spontaneous contractions decreased by 33% and oxytocin-induced contractions decreased by 30%.

The uterus is one of the smooth muscle organs that has reversible changes by ovarian hormones during pregnancy. These changes facilitate the adaptation of the uterus to the stretching caused by the growing fetus (Riemer and Heymann, 1998). The uterus has many roles in various reproductive functions, including uterine motility, sperm and embryo transport, implantation, pregnancy and labor. Disturbances in uterine contractions can cause common and important problems such as infertility, implantation problems, dysmenorrhea, endometriosis, embryonic-fetal loss or premature birth (Aguilar and Mitchell, 2010).

In this study, it was aimed to investigate the effect of omentin on uterine contractions. Therefore the effect of omentin on uterine spontaneous contractions and contractions induced by oxytocin and $PGF_{2\alpha}$ was evaluated in healthy nonpregnant rats. Thus, the limited literature in this field will contribute to the data.

MATERIALS AND METHODS

Experimental Animals

Uterine tissues isolated from 20 virgin female Wistar albino rats weighing 250-300 grams (g), approximately 5 months old, were used in the study. The rats were started to be used after a 15-day quarantine, under 12 hours of light and 12 hours of darkness, without the restriction of feed and water. The rats used in the study were taken care of at Kırıkkale University Hüseyin Aytemiz Experimental

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Research and Application Center. The study was approved by the decision of Kırıkkale University Animal Experiments Local Ethics Committee dated 18.09.2019 and numbered 2019/7 (Decision No: 42).

Chemicals and Reagents

Omentin Human E. Coli / (Biovendor, RD172100100): Dissolved in distilled water. Prostaglandin $F_2\alpha$ Tris (Sigma, P0424): Dissolved in distilled water, Oxytocin (Oksitosin®, Vetaş), Ketamin (Ketalar®, Pfizer), Xylazine (Rompun®, Bayer), Dale's solution: NaCl 154 mM, KCl 5.63 mM, $NaHCO_3$ 5.95 mM, $CaCl_2$ 1.63 mM, $MgCl_2$ 0.024 mM and Dekstroz 2.77 mM (It was dissolved in distilled water and the pH of the solution was adjusted to 7.4).

Study Design

For the anesthesia of the rats, intramuscular xylazine (10 mg/kg) followed by ketamine (50 mg/kg) administration was performed. Then the anterior abdominal wall was opened and the uterus was reached by dissection of the surrounding tissues, and the right and left cornu uteri were isolated. After this procedure, cornu uteri were taken into a petri dish containing Dale's solution at +4°C. Tissues were removed from the surrounding tissue and fat using fine-tipped scissors. The cornu uteri, which were removed from the surrounding structures, were cut to approximately 1 cm in length, and Biopac Systems MP 35 (Commat, Turkey) was suspended in the isolated organ bath containing 10 mL of Dale's solution with 1 g pre-tension. During the experiments, a gas mixture of 95% oxygen-5% carbon dioxide was applied to the tissues at 37 °C.

Uterine sections were allowed to equilibrate for 45 minutes in the isolated organ bath. During equilibration, the Dale's solution contained in the tissues was drained and added (10 mL) every 15 minutes. The protocols of the study were applied on tissues producing spontaneous contraction. The changes in the tension of the isolated tissues during the experiments in the protocols were measured with an isometric tension transmitter (transducer) and recorded on the computer.

The study was conducted using three protocols: Isolated uterine sections from the same animal were suspended in each isolated organ bath. After a 45 minute equilibration period, 10 minutes of spontaneous uterine contraction was obtained when its spontaneous contractions were balanced, and 300 µL of distilled water (the amount of distilled water was determined based on the volume of the highest concentration omentin applied to the isolated organ bath), 10, 100 and 300 ng/mL omentin (Yamawaki et al., 2010) was administered for 10 minutes. Omentin concentrations were tested on the uterine tissue, which was spontaneously contracted in the 1st protocol. In the 2nd protocol 300 µL of distilled water (control), 10, 100 and 300 ng/mL omentin were applied on the uterus contracted with 2.5 mIU/mL oxytocin (Öcal et al. 2004) and in the 3rd protocol 300 µL of distilled water (control), 10, 100 and 300 ng/mL omentin were applied on the uterus contracted with 10^{-6} M $PGF_2\alpha$ (Öcal et al. 2004).

Changes in contractions in the uterine tissues were recorded on the computer by an isometric tension transmitter. Changes in contractions were evaluated within each protocol itself. In order to better detect the changes in contractions and relaxations, the average of all contractions (mean amplitude), the highest contraction (peak amplitude) and the number of contractions (frequency) were calculated in 10-minutes period.

The frequency was calculated by counting the rhythmic contractions of the isolated uterine in 10-minutes period. The mean amplitudes were measured by calculating the average of all rhythmic contractions of the uterine (the amplitudes were calculated from the base of the trace to the peak of the trace) in 10 minutes period and expressed in terms of mg. The peak amplitude was the highest amplitude of the contractions in 10 minutes period.

Statistical Analysis

Statistical analysis of the study was done with the statistical package program "SPSS 15 for Windows". The data obtained were presented as arithmetic mean and standard error. 300 µL of distilled water contained 10, 100 and 300 ng/mL omentin frequency, mean amplitude, and peak amplitude data in all protocols were analyzed by Duncan test as One Way ANOVA posthoc test. Statistical significance was accepted as $p<0.05$.

RESULTS

The findings of the study are presented in Tables 1, 2, and 3. The frequency and amplitude (mean and peak) of the control group in spontaneous uterine contractions were not statistically different from the 10, 100 and 300 ng/mL omentin applied groups ($p>0.05$) (Table 1). The frequency and amplitude (mean and peak) of the control group in 2.5mIU/mL oxytocin induced uterine contractions were not statistically different from the 10, 100 and 300 ng/mL omentin applied groups ($p>0.05$) (Table 2). The frequency and amplitude (mean and peak) of the control group in 10^{-6}M $PGF_{2\alpha}$-induced uterine contractions were not statistically different from the 10, 100 and 300 ng/mL omentin applied groups ($p>0.05$) (Table 3). As a result, 10, 100 and 300 ng/mL omentin administered for 10 minutes did not have an effect on spontaneous, oxytocin or $PGF_{2\alpha}$-induced uterine contractility.

Table 1. Comparison of frequency, mean amplitude (mg) and peak amplitude (mg) values of control, 10 ng/mL, 100 ng/mL and 300 ng/mL omentin on spontaneous uterus contractions.

Protocols	Frequency	Mean Amplitude	Peak Amplitude
300 µL distilated water (Control) (n:6)	7.33±1.48	3239.73±930.19	3348.50±942.90
10 ng/mL omentin (n:6)	6.33±1.73	3400.60±605.39	3505.17±597.61
100 ng/mL omentin (n:6)	7.83±1.45	3474.23±795.21	3623.67±783.47
300 ng/mL omentin (n:6)	8.33±1.38	3851.63±746.84	4067.83±744.12
p	0.812	0.953	0.923

Table 2. Comparison of frequency, mean amplitude (mg) and peak amplitude (mg) values of control, 10 ng/mL, 100 ng/mL and 300 ng/mL omentin administration of on 2.5 mIU/mL oxytocin induced uterus contractions.

Protocols	Frequency	Mean Amplitude	Peak Amplitude
300 µL distilated water (Control) (n:7)	11.28±0.18	6403.19±653.54	7097.84±632.23
10 ng/mL omentin (n:6)	12.67±0.71	5161.31±699.87	5871.47±614.60
100 ng/mL omentin (n:7)	10.86±0.51	7573.49±983.86	8114.80±1079.16
300 ng/mL omentin (n:7)	10.86±0.63	5656.31±760.53	6003.49±807.88
p	0.096	0.189	0.208

Table 3. Comparison of frequency, mean amplitude (mg) and peak amplitude (mg) values of control, 10 ng/mL, 100 ng/mL and 300 ng/mL omentin applied on 10^{-6}M $PGF_2\alpha$ induced uterus contraction.

Protocols	Frequency	Mean Amplitude	Peak Amplitude
300 µL distilated water (Control) (n:7)	10.43±0.37	5047.88±444.19	5288.66±492.68
10 ng/mL omentin (n:7)	10.29±0.61	5700.72±557.52	6045.07±645.91
100 ng/mL omentin (n:7)	10.29±0.47	5449.35±365.92	5871.77±474.71
300 ng/mL omentin (n:7)	10.29±0.42	6139.64±541.99	6353.96±562.23
p	0.995	0.458	0.580

DISCUSSION AND CONCLUSION

There are many studies investigating the effects of adipokines on uterine contractions and mostly inhibitory effects are reported. According to studies, adipokines such as leptin, visfatin and apelin decrease myometrial contractility, and visfatin are the most powerful relaxant among these adipokines (AlSaif et al. 2015). Hehir and Morrison (2012) showed that apelin has a strong inhibitory effect on human myometrial contractility and this inhibition was noted for both spontaneous and agonist (oxytocin)-induced contractions in the *in vitro* model.

Kutlay et al. (2019) studied the effect of 100, 200 and 400 ng/mL concentrations of omentin at the end of the stabilization period of the hypertensive groups and the control group on isolated rat hearts; and found that omentin decreased the left ventricular diastolic pressure of the hypertensive rats. In the same study, researchers also observed that the heart rate did not change after omentin was given to normotensive rats. Kutlay et al. (2019) concluded that omentin does not play a role in the regulation of heart rate in healthy rats. Leandro et al. (2021) investigated the effect of omentin-1 on endothelial function and perivascular adipose tissue in a non-obese type 2 diabetes mellitus (T2D) animal model, in Goto-Kakizaki (GK) rats with or without a high-fat diet; and found that treatment with 18 µg/kg/day omentin for 4 weeks normalized relaxation of aortic rings induced by acetylcholine in GK rats fed a high-fat diet in this animal model of T2D. They found that omentine was ineffective in regulating sodium nitroprusside-induced endothelial-independent vasodilation in arteries mounted without perivascular adipose tissue. Kazama et al. (2014) showed that omentin prevents monocrotaline-induced pulmonary arterial pressure. Researchers determined that 18 mg/kg/day intraperitoneally administered omentin for 14 days corrected the impairment in epithelial-dependent acetylcholine relaxations caused by monocrotaline in ring-shaped intrapulmonary arterial tissues. Yamawaki et al. (2010) tried 1, 10, 100 and 300 ng/mL omentin in rat aorta for 30 minutes, and they found that especially 300 ng/mL omentin, pre-contraction with noradrenaline, decreased the contractions of the aorta with intact endothelium. This effect was found to be less in endothelial removed tissue. This relaxation was inhibited by a nitric oxide synthetase inhibitor. Yamawaki et al. (2010) reported that omentin has a relaxing effect on isolated blood vessels, and this effect is regulated by endothelial-derived NO. In this study, 10, 100 and 300 ng/mL omentin applied for 10 minutes did not affect uterine contractility. This difference was associated with the difference in the uterus and aortic tissue. Although both tissues have a smooth muscle structure; NO released from the endothelium (endothelium-derived relaxing factor) plays an important role in aortic relaxations (Rameshrad et al. 2016). Endothelium-induced relaxations have been demonstrated in arteries from many mammalian species, including humans (Buse et al. 1985). Although acetylcholine-induced relaxation in the intact isolated aorta increases the frequency and myometrial contractions without changing the phasic effect *in vitro* (Tica et al. 2011). Considering all these, the relaxation effect seen in the aortic tissue may occur in uterine tissue at higher doses and with longer incubation periods.

In a recent *in vitro* study, they found that omentin statistically reduced uterine contractions compared to normal uterine contractions in obese human myometrium after cesarean section. This decrease was not observed after oxytocin administration (Mustafa et al. 2020). Mustafa et al. (2021), tried 1 nM/L, 10 nM/L, 100 nM/L, 1 µM/L, 10 µM/L omentin and stated that omentin contractions reduced spontaneous contractions by 33% and oxytocin contractions by 30% in the pregnant uterus; They found that the relaxant response occurred at a concentration of 1 nM/L. The differences in the results in our study may due to differences in species and physiological status (in obesity and pregnancy cases where the vascular structure changes). It was stated that omentin is barely expressed in adipose tissue of mouse, showing that omentin may play a more important role in adipose biology in humans than in mice (Yang et al. 2006).

As a result, even if the expected relaxant effect of omentin has not emerged at the indicated doses, this issue will be clarified with further studies with longer incubation periods (30 minutes) at higher doses (more than 500 ng/mL). The effect of omentin on pregnant rat uterus should also be evaluated. Studies on this subject usually have focused on experimental animals and humans therefore the effects of omentin on different species especially on farm animals, should also be investigated. To investigate the effects on the reproductive system of farm animals for *in vivo* and *in vitro* systems in case of impaired health conditions will make a great contribution to

veterinary practice. The use of omentin in healthy rat isolated uterus will shed light on future studies.

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Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: C.Y., H.C.M., Design: C.Y., H.C.M., Data Collection or Processing: C.Y., H.C.M., Analysis or Interpretation: C.Y., H.C.M., Literature Search: C.Y., H.C.M., Writing: C.Y., H.C.M.

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