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Changes of Plasma Levels of Adipocytokines During 120 Hours of Fasting After Endoscopic Treatment

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Abstract

Study background: Adipocytokines are secreted from adipocytes and are important for metabolic regulation and immune response. We investigated differences in plasma adipocytokine levels between diabetes mellitus patients and non-diabetes mellitus patients during 120 hours of fasting after endoscopic treatment. We also compared changes in plasma adipocytokine levels from minimally invasive endoscopically-treated patients to those of patients treated with pancreatoduodenectomy.

Materials and Methods: Seven diabetes mellitus patients, nine non-diabetes mellitus patients, and six patients who underwent pancreatoduodenectomy were analyzed for their WBC counts, plasma levels of CRP, glucose, adiponectin, adipsin, resistin and IL-6 levels at four points. Blood samples were drawn at pre-treatment, 24 hours of fasting, 72 hours of fasting, and 120 hours of fasting after treatment. Adipocytokines were examined using an ELISA method.

Results: Plasma adiponectin levels slightly decreased during the fast, and resistin levels transiently increased at the 24-hour time point in both diabetes mellitus patients and non-diabetes mellitus patients. Plasma adipsin levels were stable. In patients who underwent pancreatoduodenectomy, both a decrease and increase of adipocytokine plasma levels occurred. There was a significant percentage decrease of plasma adiponectin and adipsin levels during the fast in patients who underwent pancreatoduodenectomy compared to those of endoscopically treated patients. In pancreatoduodenectomy patients, plasma resistin, IL-6 levels were significantly increased compared to those of endoscopically treated patients after treatment.

Conclusions: Prolonged fasting after endoscopic treatment did not influence plasma adiponectin, adipsin, and resistin levels both for diabetes mellitus patients and non-diabetes mellitus patients. The decrease of plasma adiponectin and adipsin levels, and the increase of plasma resistin and IL-6 levels were significant in patients who underwent pancreatoduodenectomy. Surgical stress may influence plasma adipocytokine levels, but 120 hours of fasting with minimal invasive stress, such as endoscopic treatment, may have no effect.

Keywords: Adiponectin; Adipsin; Resistin; IL-6; Fasting

Introduction

Adipose tissue is important, not only for storing of fatty acids, but also for secreting hormones [1]. For immune response, the adipocytes secrete proinflammatory cytokines, such as TNF-alpha, IL-6 [2]. Adipocytes regulate glucose metabolism by secreting adiponectin, adipsin, resistin [1-3]. Surgical stress causes an increase or decrease of these adipose-derived hormones [4,5]. Stress from fasting or dietary restriction can also change these hormones [6-8]. However, previous studies have failed to demonstrate if fasting influences the plasma levels of adiponectin, resistin, leptin [9,10]. In these studies, the duration of fasting was several hours to three days [9,10], which may be too short to demonstrate changes in plasma adipocytokine levels. Here, we examined the effect of five days of fasting after endoscopic treatment for gastrointestinal diseases on the plasma levels of adipocytokines for diabetes mellitus (DM) patients and non-DM patients. Minimal invasive treatment and fasting for five days may influence plasma levels of adipocytokines.

Methods

Nine non-DM patients (non-DM group) and seven DM patients (DM group) underwent endoscopic mucosal resection were investigated for their WBC counts and the plasma levels of CRP, glucose, adiponectin, adipsin, resistin, and IL-6. Glicemia was stable using several kinds of drugs in DM patients. Blood samples were drawn at four points: pre-endoscopic treatment, 24 hours of fasting, 72 hours of fasting, 120 hours of fasting after endoscopic treatment. Oral intake was prohibited in all patients during the fasting period and only intravenous fluid with 4.3% glucose were administered. Plasma samples were kept at -80°C for subsequent assays. No patients complained of abdominal pain and none had a fever after endoscopic resection. (The patients who had inflammatory manifestations after endoscopic resection were excluded from the study.) The plasma levels of adiponectin, adipsin, resistin, and IL-6 were examined by

commercial ELISA kit (R&D .Systems Inc. Minneapolis, MN). Data are also shown as the percent of pre-treatment value. For comparison, WBC counts and the plasma levels of CRP, glucose, and adipocytokines of the six patients who were underwent pancreatoduodenectomy (PD) without complication (PD group) were investigated in the same manner.

Statistical analysis was performed using Kruskal-Wallis test or Mann-Whitney's test. All analyses were conducted using the Graph Pad Prism5® statistical software package (Graph Pad Software Inc., La Jolla, CA, USA). A P value less than 0.05 was considered significant. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, and was approved by the Ethical Committee of our hospital.

Results

Comparison between the non-DM and DM groups

The patient backgrounds from each group are listed in Table 1.

	non-DM group	DM group	PD group
Age (y.o)	70.8	73.4	72.3
Sex (male/female)	05-Apr	05-Feb	03-Mar
BMI	22.3	24.5	22.3
A1c(%)	5.4	6.9[*]	5.4
Serumalbumin (g/dl)	4.1	4.1	3.4
T-cholesterol (mg/dl)	197	171	190
triglyceride (mg/dl)	105	127	103
[*]P value: 0.0027 Kruskal-Wallis test			

Table 1: Patient background from non-DM, DM, PD groups.

Blood WBC counts, and plasma CRP and glucose levels in non-DM and DM patients: In both endoscopically treated non-DM and DM-groups, WBC counts were slightly elevated at 24 hours and these values decreased 120 hours after the treatment (pre: 5285 ± 1294/μl vs. 5814 ± 1965/μl, 24 hours: 7485 ± 2440/μl vs. 7900 ± 1954/μl, 72 hours: 6125 ± 1144/μl vs. 6280 ± 1712/μl, 120 hours: 6050 ± 1011/μl vs. 5757 ± 1846/μl, for the non-DM and DM groups, respectively) (Figure 1A). Plasma CRP levels were elevated at 72 hours in both the non-DM and DM groups. However, CRP levels decreased at 120 hours (pre: 0.3 mg/dl vs. 0.3 mg/dl, 24 hours: 0.5 ± 0.23 mg/dl vs. 0.325 ± 0.05 mg/dl, 72 hours: 0.75 ± 0.21 mg/dl vs. 0.45 ± 0.21 mg/dl, 120 hours: 0.45 ± 0.21 mg/dl vs. 0.3 ± 0 mg/dl, in the non-DM and DM groups, respectively) (Figure 1B). However, there were no significant differences between the two groups. Plasma glucose levels in the DM group were higher than the non-DM group, but there were no significant differences between the two groups at all points (pre: 96 ± 5.9 mg/dl vs. 128.1 ±46.3 mg/dl, 24 hours: 104.4 ± 20.6 mg/dl vs. 127.3 ± 24.4 mg/dl, 72 hours: 99.5 ± 10.4 mg/dl vs. 105.4 ± 18.3 mg/dl, 120 hours: 106.4 ± 17.7 mg/dl vs. 111.6 ± 24 mg/dl, for the non-DM and DM groups, respectively) (Figure 1C).
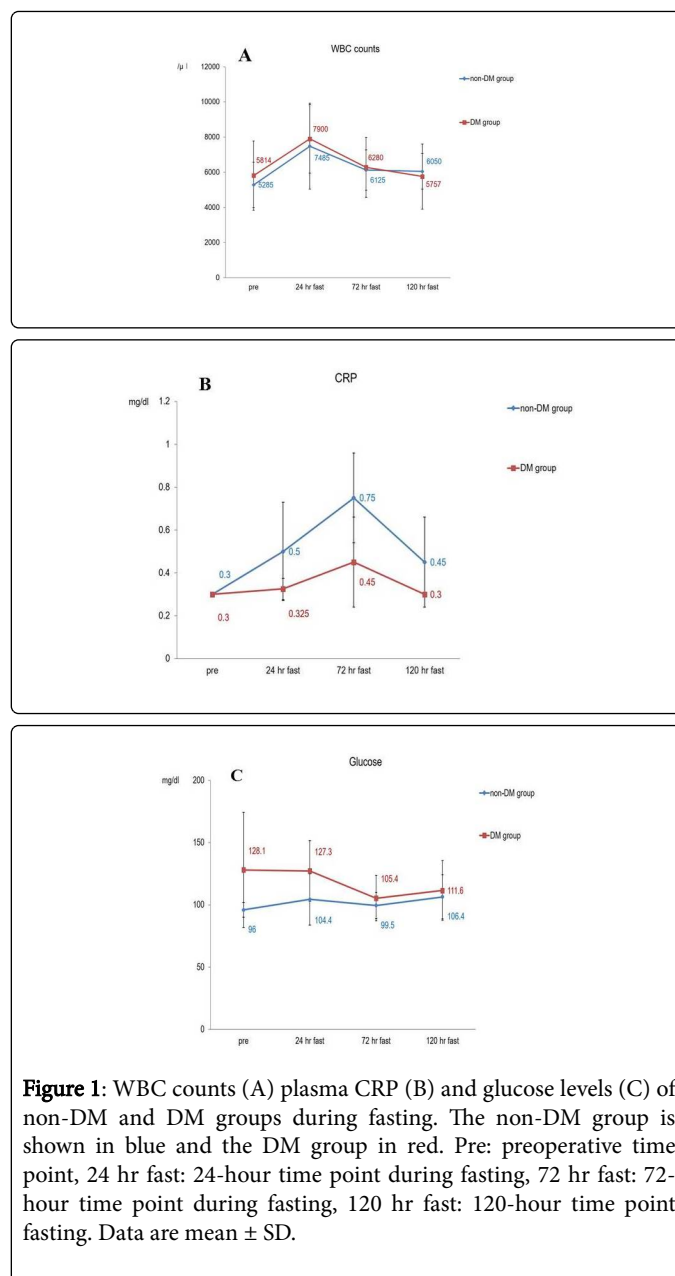

Figure 1: WBC counts (A) plasma CRP (B) and glucose levels (C) of non-DM and DM groups during fasting. The non-DM group is shown in blue and the DM group in red. Pre: preoperative time point, 24 hr fast: 24-hour time point during fasting, 72 hr fast: 72-hour time point during fasting, 120 hr fast: 120-hour time point fasting. Data are mean ± SD.

Plasma adiponectin, adipsin, resistin and IL-6 levels in the non-DM and DM groups: The plasma adiponectin levels of non-DM and DM groups decreased during the fast. The plasma adiponectin levels of the non-DM group were higher than the DM group at all points, except after 120 hours (pre: 19.6 ± 17.9 μg/ml vs 14.3 ± 11.9 μg/ml, 24 hours: 17.2 ± 17.9 μg/ml vs 15.1 ± 14.4 μg/ml, 72 hours: 11.5 ± 5.6 μg/ml vs 7.1 ± 5.2 μg/ml, 120 hours: 11.6 ± 6.1 μg/ml vs 15.9 ± 22.2 μg/ml, for the non-DM and DM groups, respectively), but there were no significant differences (Figure 2A). The plasma adipsin levels of the DM group were higher than non-DM group at all points (pre: 3.8 ± 1.4 ng/ml vs 5.5 ± 2.8 ng/ml, 24 hours: 3.2 ± 1.0 ng/ml vs 5.4 ± 3.5 ng/ml, 72 hours: 4.0 ± 1.4 ng/ml vs 5.2 ± 1.6 ng/ml, 120 hours: 4.3 ± 1.4 ng/ml vs 5.4 ± 0.9 ng/ml, for the non-DM and DM groups, respectively) (Figure 2B). The plasma resistin levels of both groups were elevated at the 24-hour time point, but decreased at the 72-hour time point. The serum levels of resistin did not differ between the two groups at all

points (pre: 13.3 ± 4.5 ng/ml *vs* 25.6 ± 31.9 ng/ml, 24 hours: 22.5 ± 9.5 ng/ml *vs* 38.5 ± 41.4 ng/ml, 72 hours: 12.8 ± 6.1 ng/ml *vs* 15.4 ± 7.1 ng/ml, 120 hours: 13.5 ± 5.4 ng/ml *vs* 14.0 ± 6.5 ng/ml, for the non-DM and DM groups, respectively) (Figure 2C). The serum levels of IL-6 were similar to those of resistin (pre: 1.8 ± 2.8 pg/ml *vs* 6.6 ± 12.1 pg/ml, 24 hours: 14.8 ± 14.8 pg/ml *vs* 18.9 ± 15.4 pg/ml, 72 hours: 11.4 ± 11.4 pg/ml *vs* 5.3 ± 2.7 pg/ml, 120 hours: 5.7 ± 8.4 pg/ml *vs* 4.5 ± 2.3 pg/ml, for the non-DM and DM groups, respectively) (Figure 2D). In the non-DM group, plasma IL-6 levels at the 24-hour and 72-hour time points were significantly increased compared to those before endoscopic treatment.
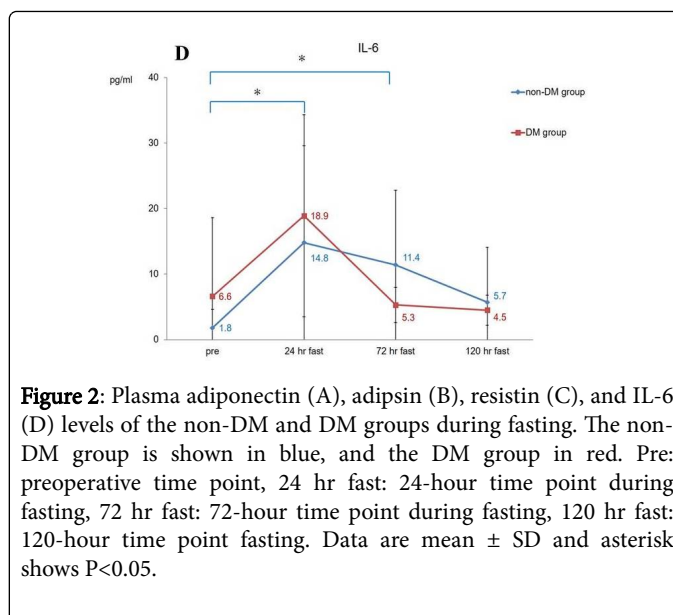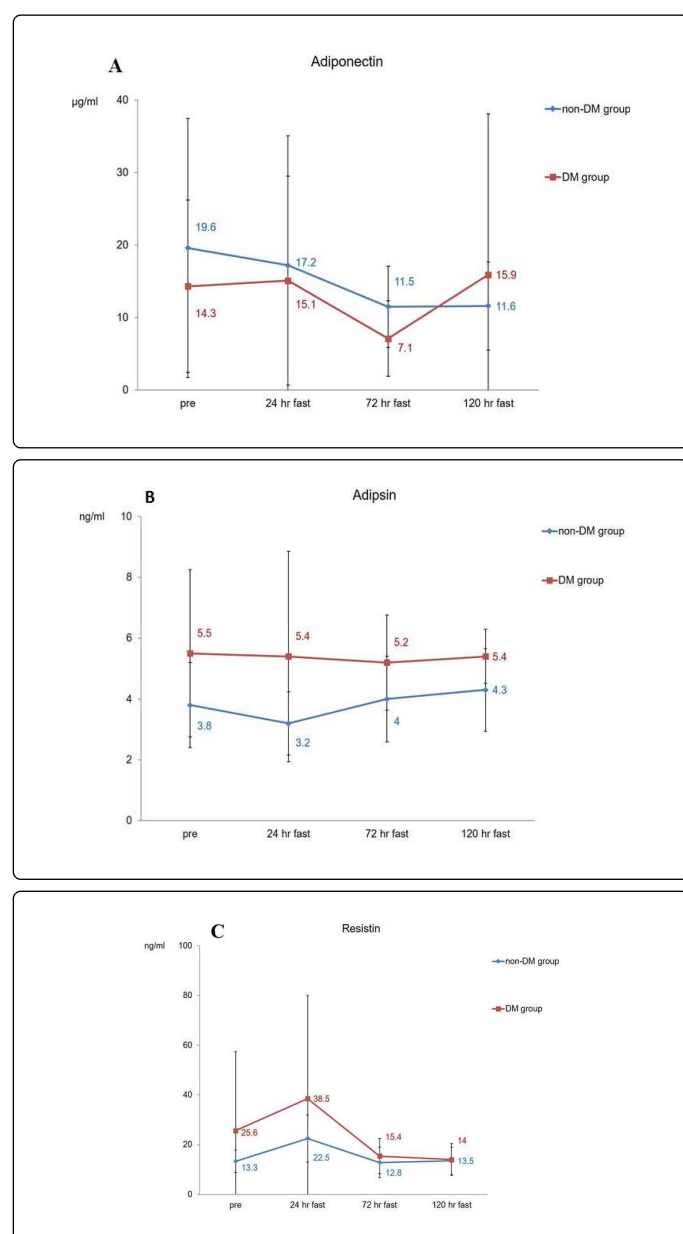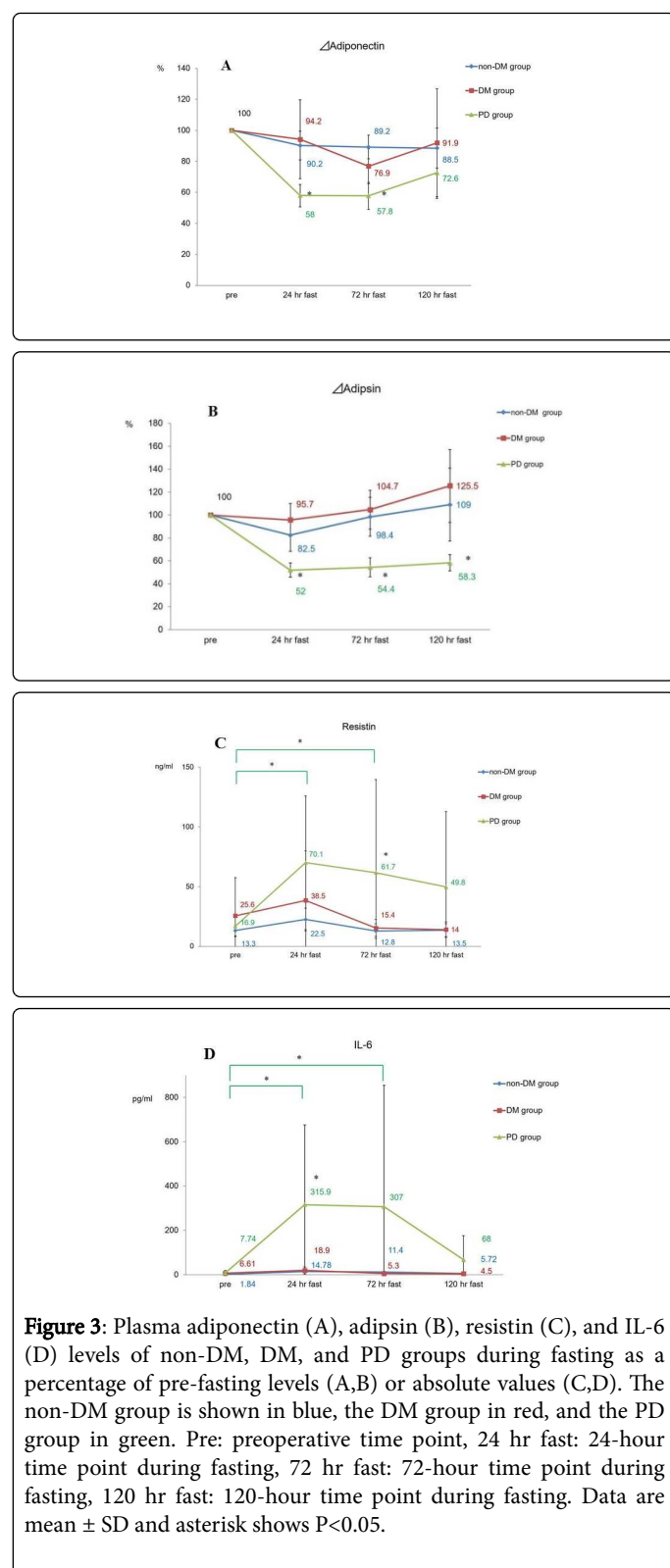

Figure 2: Plasma adiponectin (A), adipsin (B), resistin (C), and IL-6 (D) levels of the non-DM and DM groups during fasting. The non-DM group is shown in blue, and the DM group in red. Pre: preoperative time point, 24 hr fast: 24-hour time point during fasting, 72 hr fast: 72-hour time point during fasting, 120 hr fast: 120-hour time point fasting. Data are mean ± SD and asterisk shows P<0.05.

Comparison of endoscopically treated patients and PD group for WBC counts, plasma CRP, glucose, adipocytokine levels: In the PD group, WBC counts were significantly higher than those of endoscopically treated patients at the 24-hour time point. Plasma CRP levels of the PD group were also significantly higher than those of endoscopically treated patients at the 24-hour, 72-hour, and 120-hour time points. The plasma glucose level of the PD group was increased at the 24-hour time point, but there were no significant differences between endoscopically treated patients and the PD group at all points (data not shown). Plasma levels of adiponectin and adipsin were not different among the three groups during fasting. However, by comparing values as the percent of pre-treatment levels, the adiponectin levels of the PD group at the 24-hour and 72-hour time points were significantly lower compared to non-DM and DM endoscopically treated patients (24 hours: 58 ± 7.2% *vs* 90.2 ± 9.2%, 94.2 ± 25.5% P=0.0028, 72 hours: 57.8 ± 8.7 % *vs* 89.2 ± 7.6%, 76.9 ± 11.3% P = 0.005, for the PD *vs* non-DM and DM groups, respectively) (Figure 3A). In addition, the adipsin level of the PD group also significantly decreased at the 24-hour, 72-hour, and 120-hour time points (24 hours: 52 ± 6.1% vs 82.5 ± 5.3%, 95.7 ± 14.2% P=0.0016, 72 hours: 54.4 ± 8.3 *vs* 98.4 ± 10.9%, 104.7 ± 16.9% P=0.0043, 120 hours: 58.3 ± 7.1% *vs* 109 ± 19.9%, 125.5 ± 31.8% P=0.0034, for the PD *vs* non-DM and DM groups, respectively) (Figure 3B). In the PD group, the plasma level of resistin was significantly increased at the 24-hour and 72-hour time points. Compared to that of endoscopically treated patients, the resistin levels of the PD group significantly increased at the 72-hour time point (61.7 ± 77.9 ng/ml *vs* 12.8 ± 5.4 ng/ml, 15.4 ± 6.5 ng/ml, P = 0.0347, for the PD *vs* non-DM and DM groups, respectively) (Figure 3C). The plasma IL-6 level of the PD group was significantly higher than that of endoscopically treated patients at the 24-hour time point (24 hours: 315.9 ± 360.2 pg/ml *vs* 14.8 ± 14.8 pg/ml, 18.9 ± 15.4 pg/ml P=0.013, for the PD *vs* non-DM and DM groups, respectively) (Figure 3D). The plasma levels of TNF-alpha were also examined, but we did not find a significant difference among the three groups (data not shown).

Figure 3: Plasma adiponectin (A), adipsin (B), resistin (C), and IL-6 (D) levels of non-DM, DM, and PD groups during fasting as a percentage of pre-fasting levels (A,B) or absolute values (C,D). The non-DM group is shown in blue, the DM group in red, and the PD group in green. Pre: preoperative time point, 24 hr fast: 24-hour time point during fasting, 72 hr fast: 72-hour time point during fasting, 120 hr fast: 120-hour time point during fasting. Data are mean ± SD and asterisk shows P<0.05.

Discussion

Adipocytokines are secreted from adipose tissue and contribute metabolic regulation and immune response [1,2]. Adiponectin, adipsin and resistin affect glucose metabolism [1-3]. Adiponectin decreases the plasma glucose level by increasing insulin sensitivity [11,12]. Recently, adipsin was shown to stimulate insulin secretion from pancreatic beta cell [3], and resistin increases resistance to insulin sensitivity [13,14]. The influence of fasting or surgical stress on the plasma levels of these adipocytokines have been reported. Murphy *et al* [9] demonstrated that a six-hour fast did not influence serum adiponectin level [9]. Lee *et al* [10] demonstrated that 48 hours of fasting did not influence serum resistin levels [10]. Asgeirsson et al. demonstrated that serum adiponectin levels decreased at POD 3 and serum resistin levels increased at POD 1 after colectomy [4]. These results show that plasma adiponectin or resistin levels are stable during fasting. In addition, surgical stress has a greater impact on serum adiponectin and resistin levels than stress from fasting. In type 2 DM patients, plasma adiponectin levels were lower and plasma resistin levels were higher than normal patients [15,16]. This means that changes in plasma adipocytokine levels influence plasma glucose levels, and that the regulation of plasma glucose in DM patients differs from normal patients.

In this study, we investigated whether 120 hours of fasting (with 4.3% glucose administration) under minimal invasive treatment influences the plasma levels of adiponectin, adipsin and resistin, and whether there are differences between DM patients and normal patients. We did not observe a difference in plasma adipocytokine levels during fasting between DM and non-DM patients possibly because 120 hours is too short to detect changes in these adipocytokines. Alternatively, intravenous glucose administration may have influenced secretion of these adipocytokines.

The effect of surgical stress on plasma levels of adipocytokines was also evident in our study. The increase of resistin and IL-6 were remarkable, and the decrease of adiponectin and adipsin were significant. Surgical stress usually induces a release of fatty acids from adipose tissue for energy. It is known that cortisol also influences postoperative insulin resistance and hyperglycemia [17]. Plasma adipocytokines may help maintain hyperglycemia in response to surgical stress and may indicate the degree of surgical stress because resistin and IL-6 are secreted not only from the adipocytes, but also from macrophages and lymphocytes [18-20]. In conclusion, fasting does not influence plasma adipocytokine levels more than the surgical stress, and there were no significant differences in plasma adipocytokine levels during fasting conditions between DM and non-DM subjects.

Conclusions

There was no difference in adipocytokine secretion between DM and non-DM patients who fasted for 120 hours. In addition, surgical stress may have more influence on plasma adipocytokine levels compared to fasting.

Competing Interests

The authors have no potential conflict of interest.

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