Profiling of serum proteins influenced by warm partner contact in healthy couples

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Submitted: 2008-12-24 Accepted: 2009-04-04 Published online: 2009-07-15

Key words: **warm partner contact; ProteinChip; SELDI-TOF-MS; serum albumin; β_2-microgrobulin**

Neuroendocrinol Lett 2009; **30**(2):227–236 **PMID:** 19675516 NEL300209A04

Abstract

OBJECTIVES: Warm physical contact may positively influence our health and well-being; however, it has not been investigated yet whether serum proteins are influenced by warm physical contact in healthy couples. In this study, we focused on psychological and physiological effects of warm partner contact in healthy couples.

METHODS: When participants freely kissed and hugged their romantic partners, they were asked to subjectively evaluate their present emotions. Furthermore, changes of serum proteins were determined by using ProteinChip surface enhanced laser desorption/ionization-time-of-flight-mass spectrometry (SELDI-TOF-MS). We characterized these proteins by using biochemical techniques combined with gel filtration high performance liquid chromatography (HPLC), reverse-phase HPLC, and sequencing analyses.

RESULTS: Romantic couples became happier and less irritated after kissing and hugging. Accompanying these psychological changes, SELDI-TOF-MS indicated that the intensities of 66-k Da, 11.7-k Da, and 5.9-k Da serum proteins were increased. These proteins were identified as serum albumin and β_2-microglobulin, and probably fibrinogen fragment. The feeling of happiness positively correlated and the feeling of irritation negatively correlated with intensities of serum albumin and β_2-microglobulin.

CONCLUSION: These results suggest that psychological stress may be reduced and we may feel happiness when we kiss and hug a romantic partner. Furthermore, these results also suggest that warm partner contact influences peripheral circulating proteins, more importantly, may promote health and well-being.

ORIGINAL ARTICLE

INTRODUCTION

When we see the person we are in love with, our heart may be filled with fortunate feelings. We may experience feelings of elation, and those feelings can subjectively put us 'on top of the world'. Romantic love may be a positive psychological event (Planalp *et al*., 2006) and previous studies indicated that participants who fell in love showed significant improvement of psychological states, such as self-efficacy and self-esteem (Aron *et al*., 2006). Furthermore, when in love, circulating levels of oxytocin, a major biochemical player in making physiological states of love, are increased, and this inhibits activation of the HPA axis resulting in a reduction of physiological stress (Esch & Stefano 2005a; 2005b; Stefano & Esch 2005). Warm physical contact such as kissing and hugging may be induced by love and may be psychologically-positive social interaction (Aron *et al*., 2006; Planalp *et al*., 2006; de Chateau & Wiberg 1977; Kimata, 2003; Grewen *et al*., 2003). When mothers kissed their infants, these infants smiled more often and cried less frequently (de Chateau & Wiberg 1977). Recent study has indicated that warm partner contact reduced allergic skin wheal responses and blood concentrations of nerve growth factor (NGF), which may activate mast cells, eosinophils, and neutrophils, in patients with allergic rhinitis or atopic dermatitis (Kimata, 2003). Recent study has also indicated that warm partner contact before stress attenuates cardiovascular reactivity in healthy couples (Grewen *et al*., 2003). Based on these previous findings, romantic love and warm physical contact may have beneficial effects on psychological and physiological aspects in healthy couples. However, to our knowledge, it has not been investigated yet whether serum proteins are influenced by warm physical contact in healthy couples.

Recently the surface enhanced laser desorption/ionization-time-of-flight (SELDI-TOF) ProteinChip has been introduced (Kozak *et al*., 2005; Oh *et al*., 2005; Lakhan, 2006; Novikova *et al*., 2006; Lewczuk *et al*., 2004; Sanchez *et al*., 2004). This technology utilizes affinity surfaces to retain adherent proteins based on their physical or chemical characteristics, which is then followed by direct analysis using TOF-mass spectrometry (MS). SELDI-TOF-MS allows users to generate protein expression data rapidly from a large number of samples and has been used increasingly to identify diagnostic biomarkers of cancer (Kozak *et al*., 2005; Oh *et al*., 2005), mental illness (Lakhan, 2006; Novikova *et al*., 2006), and neurological disorders (Lewczuk *et al*., 2004; Sanchez *et al*., 2004). Therefore, SELDI-TOF-MS may be useful to identify changes in several serum proteins after warm partner contact in healthy couples. In this study, we focused on psychological and physiological effects of warm partner contact in healthy couples, and changes of serum proteins were determined by using ProteinChip SELDI-TOF-MS when participants freely kissed and hugged their romantic partners.

MATERIAL AND METHODS

Participants

Sixteen healthy volunteers (eight romantic couples; eight males and eight females) participated in the study. The age range was 21 to 38 years. One couple did not participate in the control condition. All the participants provided written informed consent in accordance with the Declaration of Helsinki. The participants received no medication during the experimental period. They were requested to evaluate the feeling of romantic love for their romantic partner by using passionate love scale (PLS) (Hatfield & Sprecher, 1986) (example items: "Sometimes I can't control my thoughts; they are obsessively on___"; I would rather be with ___ than anyone else"). Five participants were evaluated as "extremely passionate", seven participants were evaluated as "passionate", and four participants were evaluated as "average"; therefore, all the couples might be considered to have relatively-passionate love. This study was approved by the Human Studies Committee of Aichi Medical University.

Experimental procedure

Each couple entered an experimental room, following which participants were given instructions prior to commencement of the experiment. The couple was instructed not to eat and drink in the experimental session. In the warm contact condition, participants were first requested to evaluate the present mood state and the first blood sample was obtained. They then freely kissed and hugged their romantic partner, not had an intercourse, for 1 hour in a room with closed doors. After warm contact session, second blood sample was obtained and the present mood state was evaluated. In order to evaluate whether participants really kissed and hugged their partners, the participants were requested to subjectively evaluate by rating each of the following three questions on a scale of 1 (not at all) to 7 (Yes, extremely). Did you kiss and hug your partner very much? (contact); Did you evoke much love? (love); Did you feel the partner's love? (love). The average value of the rating score of contact was 5.25 ± 0.39 and the average value of the rating score of love was 10.75 ± 0.65. Because both values were higher than the neutral values (4 (contact) and 8 (love)), warm partner contact was performed much in the warm contact session.

In the control condition, participants were first requested to evaluate the present mood state and first blood sample was obtained. Then, one person remained in the experimental room, another person moved to another experimental room, and they read a book separately for 1 hour in a room with closed doors. The content of books did not contain romance. After reading session, second blood sample was obtained and the present mood state was evaluated. The order of two conditions was counterbalanced across the couples and there was at least 2 weeks interval between two conditions.

Measurement of mood states

To evaluate the mood states of the participants, they were asked to subjectively evaluate their present emotions by rating each of the following nine questions on a scale of 1 (not at all) to 7 (Yes, extremely). Do you feel peaceful at present? (pleasantness); do you feel uneasy at present? (anxiety); do you feel tired at present? (fatigue); do you feel highly energetic at present? (vigor); are you well at present? (pleasantness); are you relaxed at present? (relaxation); do you feel refreshed at present? (vigor); are you irritated at present? (irritation); do you feel happy at present? (happiness). The mood state rating scores were calculated with respect to each criterion (pleasantness, vigor, anxiety, fatigue, relaxation, irritation, and happiness), and the mood states before and after warm partner contact or reading a book were assessed as described previously (Matsunaga *et al*., 2008a). We selected these criteria based on the results of a factor analysis of the Japanese version of the profile of mood states (POMS) (Yokoyama *et al*., 1990) because the original POMS scale included too many criteria (65 items) for use in this experimental session. Specifically, in a preliminary study, we requested 363 undergraduates to fill in the POMS; we then conducted a factor analysis on the data by using the maximum likelihood method. We selected the six criteria mentioned above since they had eigenvalues above 1.0. Of these, the first (pleasantness) and second (vigor) criteria had particularly high eigenvalues; therefore, we framed 2 questions each to assess pleasantness and vigor and 1 question for each of the other criteria. In addition, we considered that the feeling of happiness may be evoked in the warm contact condition; therefore, the seventh criterion, happiness, was added to the POMS.

Analysis of serum proteins with ProteinChip SELDI-TOF-MS

Blood samples were collected in serum-separator tubes and centrifuged at 3,000 × *g* for 10 min; the serum was separated and then stored at –80°C until analysis. Difference mapping analysis of serum proteins was performed on ProteinChip Array (Bio-Rad Laboratories, Inc., Hercules, CA). The types of arrays were strong anion exchange (Q10) and weak cation exchange (CM10) PoteinChip Arrays. Prior to sample loading, Q10 and CM10 arrays were equilibrated with 10 µl of binding buffers (50 mM Tris-HCl, pH 8.6; or 100 mM sodium acetate, pH 4; respectively) and 5 µl of serum was loaded onto the arrays. The arrays were incubated in a humid chamber for 30 minutes at ambient temperature and then washed three times with binding buffers. Washed arrays were rinsed two times with distilled water. After air-drying, 1 µl aliquot of saturated sinapinic acid (dissolved in 50% acetonitrile containing 0.5% trifluoroacetic acid) was added to each spot twice and the arrays were air dried. The ProteinChip Arrays were analyzed by ProteinChip Reader (Bio-Rad) and the data were analyzed by ProteinChip Software (Bio-rad). All data were normalized by total ion current normalization function according to the software instructions. The analyzed mass ranges from 4,000 to 1,00,000 Da. The peaks selected were then analyzed by Biomarker Wizard included in ProteinChip Software.

Purification and identification of target proteins

To estimate the isoelectric point (pI) of target proteins, we used on-chip analysis using different pH buffer. Prior to sample loading, Q10 and CM10 arrays were equilibrated with 10 µl of various binding buffers (100 mM sodium acetate, pH 4, pH 5, and pH 6; 100 mM HEPES, pH 7; 50 mM Tris-HCl, pH 8, pH 8.6, pH 9, and pH 10), 5 µl of serum was loaded onto the arrays, and they were analyzed using similar method described above.

To purify the target proteins, following biochemical techniques were used. Prior to sample loading, ProteinChip Q spin column (Bio-rad) was equilibrated with binding buffer (50 mM Tris-HCl, pH 8.6) and serum diluted twofold by binding buffer was loaded onto the column. The column fraction was eluted by 200 mM NaCl and subjected to gel filtration high-performance liquid chromatography (HPLC) using a Bio-Sil SEC 125-5 HPLC column (300 mm × 7.8 mm, Bio-Rad). The HPLC column was eluted with an isocratic gradient of ammonium acetate buffer (100 mM ammonium acetate, pH 7.0) at a flow rate of 1 ml/minute. Gel filtration standard (Bio-rad) were used as reference standard for this chromatography (Thyroglobulin: molecular mass 670,000 Da; γ-globulin: 158,000 Da; ovalbumin: 44,000 Da; myoglobin: 17,000 Da; vitamin B_{12}: 1,350 Da). After fractionating samples according to a molecular mass, the collected fraction was concentrated by freeze-drying and then subjected to reverse-phase HPLC using TSKgel ODS-120T column (150 mm × 4.6 mm, Tosoh Corporation, Tokyo, Japan). The reverse-phase HPLC column was eluted with 60 minutes linear gradient of 0.1% TFA and 0.1% TFA/100% acetonitrile at a flow rate of 1 ml/minute. The peaks of target proteins were collected, and analyzed by a protein sequencer (Procise 492, Applied Biosystems Japan, Tokyo, Japan). The amino acid sequence obtained was compared with that in the NCBI database (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Statistical analyses of self-reported and physiological data

Results were expressed as the mean ± SEM. The psychological and physiological indices were compared using two-way repeated measures ANOVA [condition (control vs. warm contact) × period (before vs. after)] followed by paired t tests. Furthermore, Pearson correlation coefficients were computed between the values of the psychological and physiological indices to examine the relationships between mood states and endocrine activities.

Table 1: Self-rating of mood states such as pleasantness, vigor, anxiety, fatigue, relaxation, irritation, and happiness in control and warm contact conditions.

	CONTROL		WARM CONTACT	
Criterion	**Before**	**After**	**Before**	**After**
Pleasantness	7.86 ±0.28	8.64 ±0.34	9.63 ±0.55	11.38± 0.64
Vigor	7.36 ±0.46	7.71 ±0.49	8.44 ±0.65	10.06 ±0.59
Anxiety	2.79 ±0.41	2.57 ±0.34	2.50 ±0.40	1.81 ±0.36
Fatigue	4.07 ±0.44	3.14±0.36	3.50 ±0.48	2.81 ±0.44
Relaxation	4.14 ±0.21	4.43 ±0.36	4.69 ±0.35	5.50 ±0.39
Irritation	2.93 ±0.34	2.79 ±0.35	2.38 ±0.43	1.19±0.10*
Happiness	3.93 ±0.71	3.93 ±0.71	4.75 ±0.23	5.69 ±0.33*

Each result represents the mean ± SEM rating score (control: n = 14 samples; warm contact: n = 16 samples). *$p < 0.05$ versus before warm partner contact, as determined by two-way ANOVA followed by paired t tests.

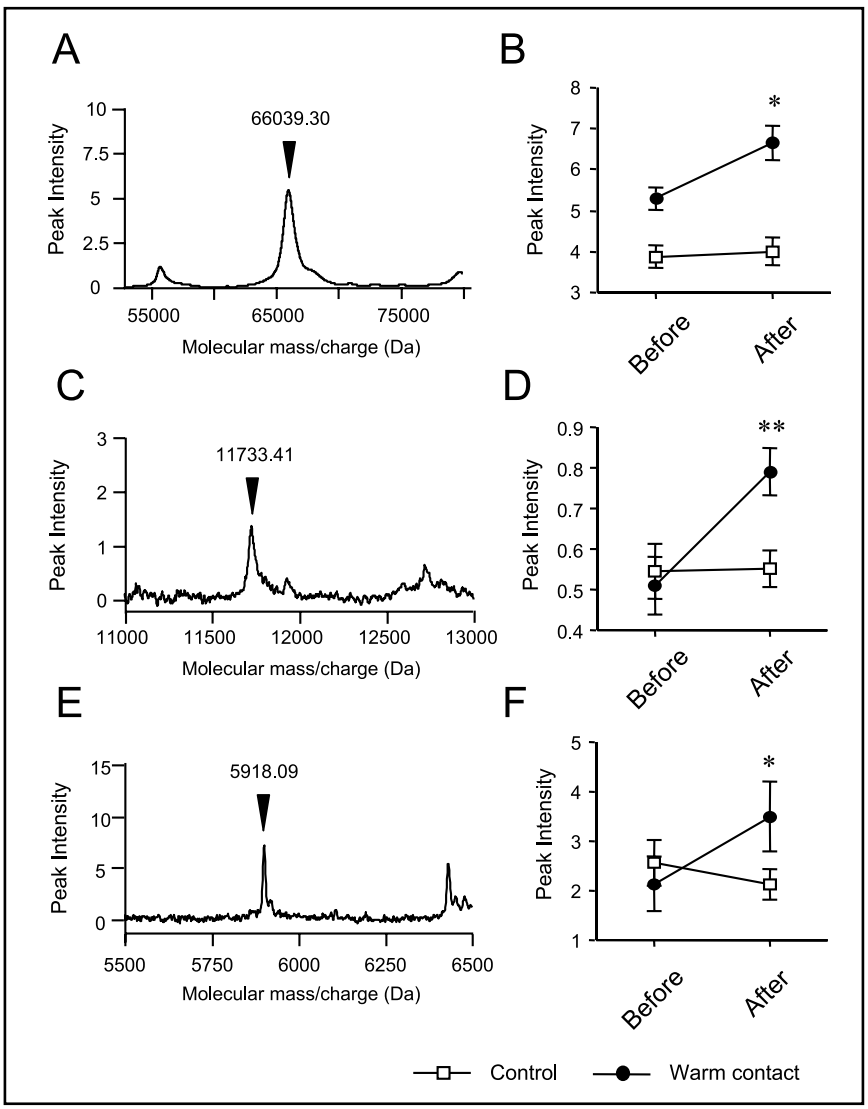

Figure 1. Serum protein profiles before and after warm partner contact. Each *point* and *vertical line* represents the mean ± SEM intensity (control: n = 14 samples; warm contact: n = 16 samples). (A) Protein profile detected by SELDI-TOF-MS with Q10 ProteinChip Array. The *arrowhead* indicates M_1 protein peak. (B) Change in the intensity of M_1 after warm contact. *$p < 0.05$ versus before warm contact by two-way repeated measures ANOVA, following the paired t test. (C) Protein profile detected by SELDI-TOF-MS with CM10 ProteinChip Array. The *arrowhead* indicates M_2 protein peak. (D) Change in the intensity of M_2 after warm contact. **$p < 0.01$ versus before warm contact by two-way repeated measures ANOVA, following the paired t test. (E) Protein profile detected by SELDI-TOF-MS with Q10 ProteinChip Array. The *arrowhead* indicates M_3 protein peak. (F) Change in the intensity of M_3 after warm contact. *$p < 0.05$ versus before warm contact by two-way repeated measures ANOVA, following the paired t test.

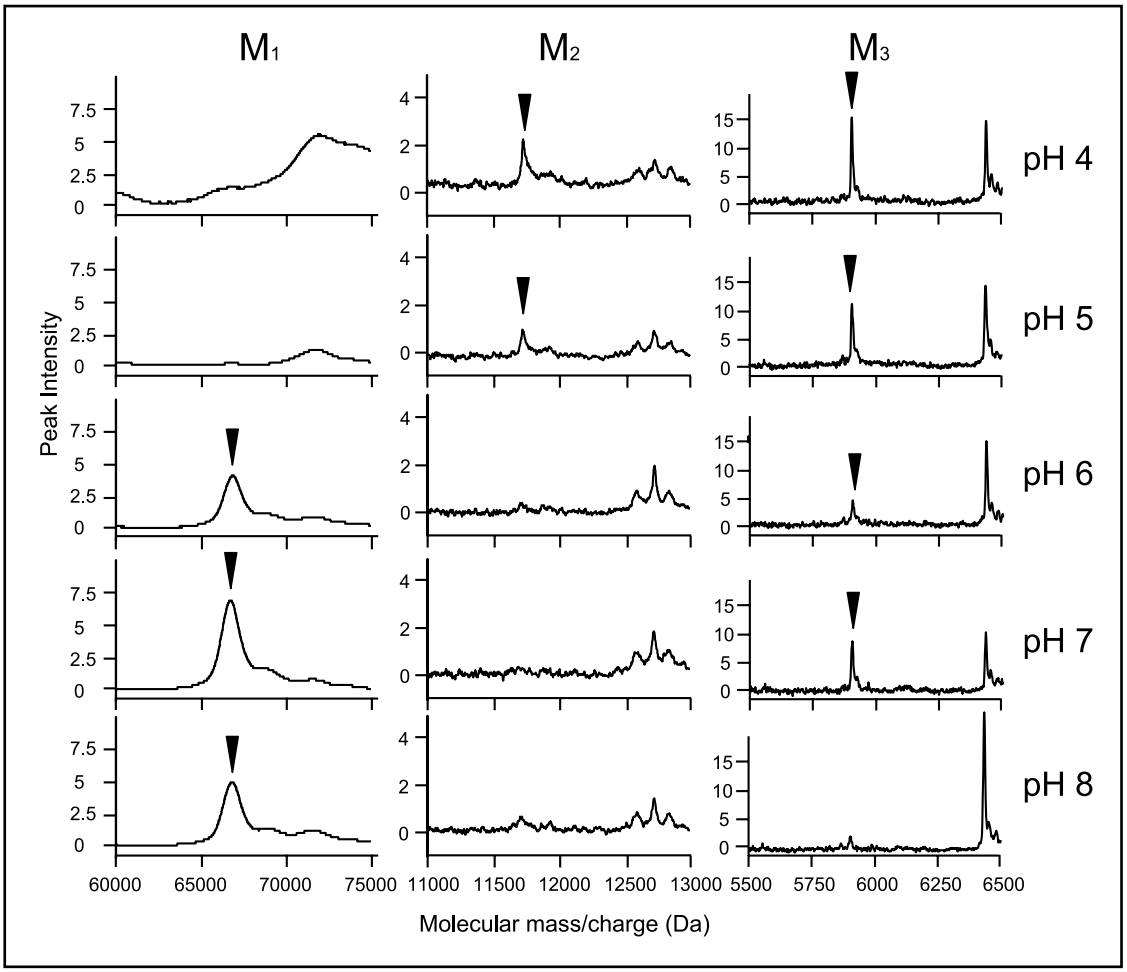

Figure 2. Protein profiles with different buffer conditions. The *arrowheads* indicate the protein peaks of interest. Q10 ProteinChip Array was used in the analysis of M_1 and CM10 ProteinChip Array was used in the analyses of M_2 and M_3

RESULTS

Psychological response

In order to assess the changes in mood states accompanying warm partner contact, the participants were asked to rate the present mood states by seven-scale rating for seven different mood states, namely, pleasantness, vigor, anxiety, fatigue, relaxation, irritation, and happiness (Table 1). ANOVAs revealed significant interactions between the condition (control or warm contact) and period (before or after) for the rating scores of irritation ($F(1,28) = 4.74$, $p < 0.05$) and happiness ($F(1,28) = 3.29$, $p < 0.05$). No significant interactions between the condition and period were observed for the rating scores of pleasantness ($F(1,28) = 1.66$), vigor ($F(1,28) = 2.26$), anxiety ($F(1,28) = 1.02$), fatigue ($F(1,28) = 0.20$), and relaxation ($F(1,28) = 0.81$). Further analyses using the paired t test revealed that the rating score for irritation decreased significantly ($df = 15$, $t = 2.97$, $p < 0.01$) and the rating score for happiness increased significantly ($df = 15$, $t = -2.39$, $p < 0.05$) after warm partner contact. In addition, we analyzed potential sex differences in changes in irritation and happiness; however, no significant interactions between the sex (male or female) and period were observed for the rating scores of irritation ($F(1,14) = 0.59$) and happiness ($F(1,14) = 0.02$). These results indicated that romantic couples became happier and less irritated after warm partner contact.

SELDI profiling of serum proteins

We analyzed serum protein profiles using Q10 ProteinChip Array, to which negative-charged proteins are bound, and CM10 ProteinChip Array, to which positive-charged proteins are bound. Following baseline subtraction and normalization using total ion current, peaks present in all of the samples were labeled and clustered automatically. Then, the peak intensity values of differentially expressed peaks identified in all samples in the 4.0- to 100-k Da mass ranges were analyzed. In the Q10 Array, an ANOVA revealed significant interaction between the condition and period ($F(1,28) = 4.19$, $p < 0.05$) for the intensity of peak M_1 (measured mass: 66039.30 Da; Fig. 1A and 1B). Further analyses using the paired t test revealed that intensity of peak M_1 significantly increased only in the warm contact condition ($df = 15$, $t = -1.36$, $p < 0.05$; Fig. 1B). In the CM10 Array, ANOVAs revealed signifi-

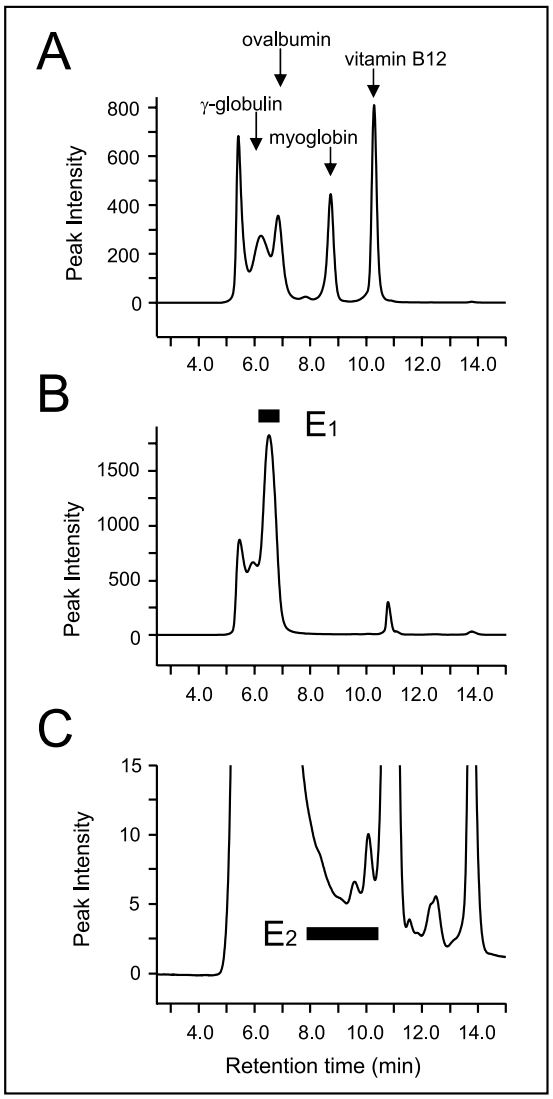

Figure 3. Typical gel filtration HPLC chromatograms of standard proteins (A) and serum sample (B, C). Magnified chromatogram revealed the elution of small proteins (C). The *arrows* indicate the protein peaks of standard proteins. The *bars* indicate the eluate that we collected.

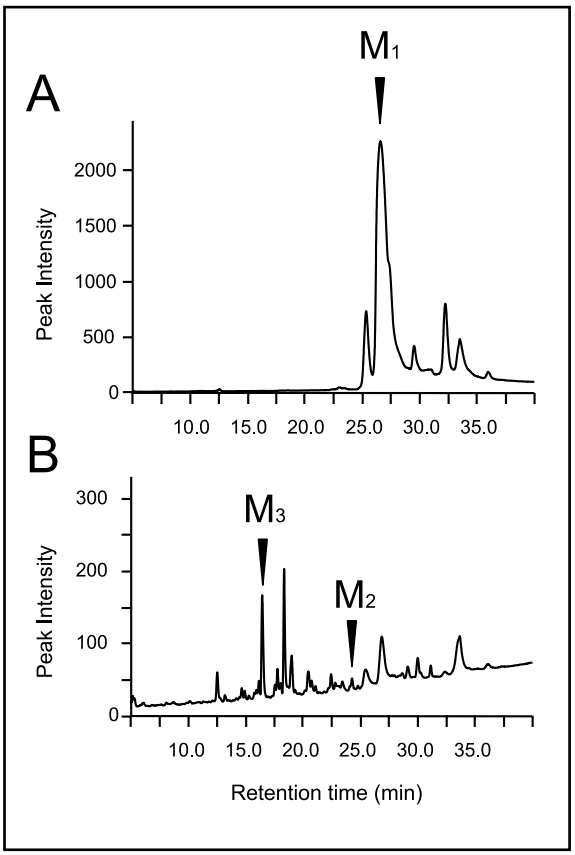

Figure 4. Typical reverse-phase HPLC chromatograms of E_1 sample (A) and E_2 sample (B). The *arrows* indicate the protein peaks containing M_1 (A), M_2 (B), and M_3 (B).

cant interactions between the condition and period for the intensities of peaks M_2 (measured mass: 11733.41 Da; $F(1,28) = 7.76$, $p < 0.01$; Fig. 1C and 1D) and M_3 (measured mass: 5918.09 Da; $F(1,28) = 5.17$, $p < 0.05$; Fig. 1E and 1F). Further analyses using the paired t test revealed that both intensities of peaks M_2 ($df = 15$, $t = -2.78$, $p < 0.01$; Fig. 1D) and M_3 ($df = 15$, $t = -2.19$, $p < 0.05$; Fig. 1F) significantly increased only in the warm contact condition. In addition, we analyzed potential sex differences in changes in M_1, M_2, and M_3; however, no significant interactions between the sex and period were observed for changes in M_1 ($F(1,14) = 0.02$), M_2 ($F(1,14) = 0.37$), and M_3 ($F(1,14) = 0.20$). These results indicated that serum protein M_1, M_2, and M_3 were increased in both sexes after warm partner contact.

Purification of target proteins

We then attempted to identify the target serum proteins. First, in order to estimate pI value of three protein candidates, we used on-chip analysis using different pH buffer. The peak of a 66-k Da protein M_1 increased at pH 6.0 condition, indicating that the pI value of M_1 may be between 5 and 6 (Fig. 2). The peak of a 11.7-k Da protein M_2 disappeared at pH 6.0 to 7.0 condition, indicating that the pI value of M_2 may be between 5 and 7 (Fig. 2). The peak of a 5.9-k Da protein M_3 disappeared at pH 8.0 condition, indicating that the pI value of M_3 may be between 7 and 8 (Fig. 2).

Based on the estimated pI values of protein candidates, it was considered that these proteins may be negative-charged at pH 8.6 condition; therefore, the serum sample was rough-purified using ProteinChip Q column at pH 8.6 condition. Subsequently, gel filtration high performance liquid chromatography (HPLC) was performed. Elution time of standard protein γ-globulin (158-k Da) was 6.2 min and that of ovalbumin (44-k Da) was 6.8 min (Fig. 3A); therefore, we collected the protein peak eluted at 6.5 min (E_1: 66-k Da protein M_1 may be contained) (Fig. 3B). Furthermore, elution time of standard protein myoglobin (17-k Da) was 8.7 min and that of vitamin B_{12} (1.3-k Da) was 10.2 min (Fig.

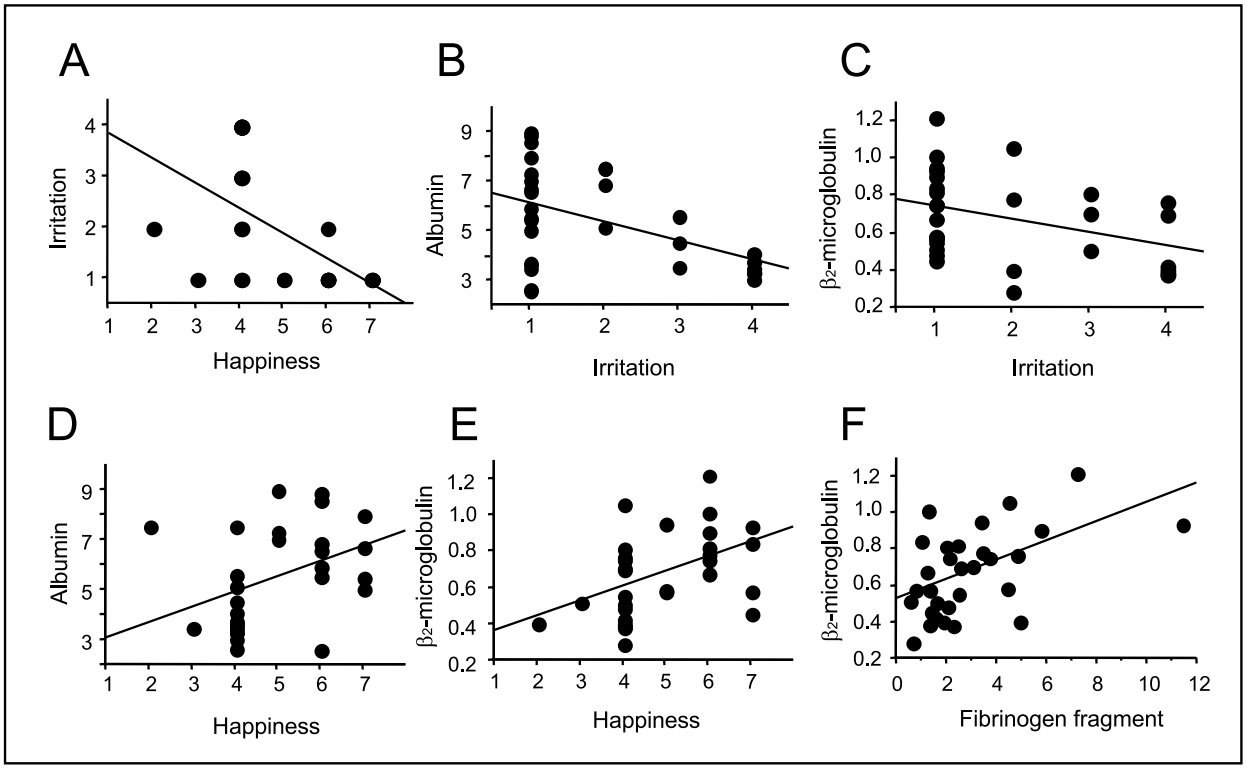

Figure 5. Scatterplots of the feelings of irritation and happiness (A), the feelings of irritation and the intensity of albumin in the serum (B), the feelings of irritation and the intensity of β_2-microglobulin in the serum (C), the feelings of hapiness and the intensity of albumin in the serum (D), the feelings of hapiness and the intensity of β_2-microglobulin in the serum (E), and the intensities of β_2-microglobulin and putative fibrinogen fragment in the serum (F) after control and warm contact sessions (n = 30 samples).

3A); therefore, we collected eluates from 8.0 to 10.5 min (E_2: 11.7-k Da protein M_2 and 5.9-k Da protein M_3 may be contained) (Fig. 3C). Then, samples of E_1, and E_2 were subjected to the reverse-phase HPLC. Typical reverse-phase HPLC chromatograms of E_1 and E_2 are shown in Fig. 4A and 4B, respectively. The corresponding mass spectrum revealed protein peaks containing M_1 (retention time: 26.6 min; Fig. 4A), M_2 (retention time: 23.4 min; Fig. 4B), and M_3 (retention time: 16.4 min; Fig. 4B).

Sequence determination

We subjected the purified protein peaks to Edman degradation analysis. As a result, it was indicated that N-terminal amino acid sequence of M_1 was DAHKSEVAHR, that of M_2 was IQRTPKIQV, and that of M_3 was DSGEGDFLAE. By using database search of these amino acid sequences, M_1 was identified to serum albumin (gi|547232; 66473 Da, pI 5.66) and M_2 was identified to β_2-microglobulin (gi|179316; 11731 Da, pI 6.08). It was also indicated that the sequence of M_3 was corresponded to fibrinogen α chain (gi|223918; 49264 Da); however, the molecular mass of M_3 was much smaller than fibrinogen α chain. The molecular mass of N-terminal 54-residue fragment of fibrinogen α chain was calculated as 5913 Da; therefore, it is possible that M_3 may be the fragment of fibrinogen α chain.

Correlations among mood states and endocrine activities

Finally, to examine the associations among the influenced psychological and physiological indices such as the feelings of irritation and happiness, the intensities of serum albumin, β_2-microglobulin, and fibrinogen fragment, in the serum, the correlations among the values after experimental procedure (both control and warm contact conditions: n = 30 samples) were computed for the entire sample set. The analyses indicated that the feeling of irritation was negatively correlated with the feeling of happiness (Fig. 5A; $r(30) = -0.521$, $p < 0.01$), intensity of serum albumin (Fig. 5B; $r(30) = -0.475$, $p < 0.01$), and intensity of β_2-microglobulin (Fig. 5C; $r(30) = -0.377$, $p < 0.05$). The feeling of happiness was positively correlated with intensity of serum albumin (Fig. 5D; $r(30) = 0.399$, $p < 0.05$), and intensity of β_2-microglobulin (Fig. 5E; $r(30) = 0.456$, $p < 0.01$). The intensities of β_2-microglobulin and fibrinogen fragment were also positively correlated (Fig. 5F; $r(30) = 0.522$, $p < 0.01$).

DISCUSSION

The present study aimed to reveal psychological and physiological effects of warm partner contact in healthy couples. When the participants kissed and hugged their romantic partners, they subjectively reported becoming

happier and less irritated. In order to reveal the change in serum proteins in the warm contact condition, we performed ProteinChip SELDI-TOF-MS analysis. This analysis indicated that 66-k Da protein M_1, 11.7-k Da protein M_2, and 5.9-k Da protein M_3 increased only in the warm contact condition. Using biochemical techniques combined with gel filtration HPLC, reversed-phase HPLC, and sequencing analyses, we identified M_1 as serum albumin, M_2 as β_2-microglobulin, and M_3 as possibly fibrinogen fragment. The feeling of happiness positively correlated and the feeling of irritation negatively correlated with intensities of serum albumin and β_2-microglobulin in the serum. These results indicate that psychological stress may be reduced and the feeling of happiness may be increased when we kiss and hug a romantic partner. Previous studies have been indicated that romantic love may be associated with stress reduction (Esch & Stefano 2005a; 2005b; Stefano & Esch 2005) and successful positive interaction may evoke positive emotions such as happiness (Aron *et al*., 2006; Planalp *et al*., 2006); therefore, these results may be reliable.

In connection with the feeling of happiness, circulating levels of several endocrine indices such as serum albumin, β_2-microglobulin, and fibrinogen fragment were also elevated. Albumin, which is produced in the liver, is an abundant plasma protein that accounts for about 60% of the plasma proteins in humans (Koplik *et al*., 2003). Albumin is essential for maintaining the osmotic pressure and also acts as a plasma carrier of non-esterified fatty acids, a multitude of toxic metabolites, hormones etc. Interestingly, recent study has indicated that albumin relate to emotional stress (Koplik *et al*., 2003). In stress conditions, significant reduction of albumin concentration is observed; however, prior administration of regulatory peptide such as Semax and delta sleep-inducing peptide, which modulate stress sensitivity, led to the absence of the decrease in the albumin. The present study indicated that albumin was negatively correlated with emotional stress, the feeling of irritation. One reason why albumin and emotional stress are related may be because stress influences liver function. In the stress situation, central corticotropin-releasing factor (CRF) is increased and accelerates sympathetic nervous function (Taché *et al*., 2004). Central administration of CRF has decreased hepatic blood flow and worsened carbon tetrachloride-induced acute liver injury (Nakade *et al*., 1998; Yokohama *et al*., 1999). Therefore, stress induces the elevation of central CRF level, central CRF decreases liver function, and consequently albumin is also reduced. Furthermore, the present study also indicated the positive correlation between the feeling of happiness and serum albumin. Recent study has indicated that probiotic feeding improved nutritional status and may contribute to suppressing infections by improving immunological status in the elderly, contributing to improvement in their quality of life (Fukushima *et al*., 2007). The blood phagocytic activity increased with probiotic feeding in the elderly subjects who had low initial activity, and the increase in serum albumin has also been demonstrated (Fukushima *et al*., 2007). Moreover, previous observations have suggested that serum albumin is an efficient scavenger of free radicals, which causes oxidative damage to the body (Soriani *et al*., 1994; Roche *et al*., 2008). Therefore, there may be the positive association between positive psychological and physiological states and serum albumin; suggesting that the mood and physical states of participants may became better by means of the serum albumin activity after warm partner contact. This finding suggests the possibility that serum albumin may be used as a biomarker of positive psychological and physiological states.

β_2-microglobulin is a component of major histocompatibility complex (MHC) class I molecules, which are present on almost all cells of the body (de Moraes-Pinto *et al*., 1999; Jacob *et al*., 2002; Garver-Apgar *et al*., 2006). MHC class I molecules are known to play an important role in the immune system. MHC class I molecules are expressed on the surface of most nucleated cells, and participate in the presentation of viral and tumour cell-derived peptide molecules to the cytotoxic T lymphocytes, a subgroup of lymphocytes and are important components of the adaptive immune response (de Moraes-Pinto *et al*., 1999). The natural turnover of the MHC class I gives rise to the release of β_2-microglobulin into plasmatic fluids and increased concentrations of β_2-microglobulin have been found in viral infections (de Moraes-Pinto *et al*., 1999); therefore, the increase of β_2-microglobulin means the activation of adaptive immune system. Furthermore, plasma fibrinogen, the principal protein of vertebrate blood clotting, is a 340-k Da glycoprotein synthesised in the liver by hepatocytes and megakaryocytes, and is known to be an inflammatory marker (Steptoe *et al*., 2005). Although we could not perfectly determine the molecule M_3, if M_3 was truly fibrinogen fragment, it is possible that the increase of M_3 also means the activation of immune functions. Supporting this hypothesis, this study demonstrated the positive correlation between β_2-microglobulin and fibrinogen fragment levels in the serum. Importantly, previous study has indicated that positive social interactions may promote wound healing in monogamous California mice, *Peromyscus californicus* (Martin *et al*., 2006). It is well known that the binding of fibrinogen to platelets is an important part of wound healing (Francis 2001) and lymphocytes are also associated with wound healing (Schäffer & Barbul, 1998), therefore, it is possible that warm partner contact may enhance the immune functions and the ability of wound healing by means of activations the MHC and fibrinogen functions.

The neural network that increases serum albumin, β_2-microglobulin, and fibrinogen levels is still under speculation. We have recently demonstrated that central nervous, endocrine, and immune systems are interrelated while we evoke positive emotions, and

attraction for a favorite person can activate the immune function, the activity of peripheral circulating natural killer (NK) cells (Matsunaga *et al*., 2008b), a subgroup of lymphocytes essential to the innate immune defense against virus-infected cells, bacteria, and tumor cells (Vivier *et al*., 2004). Dopamine is known to play an important role in the expression of positive emotions (Aron *et al*., 2005; Bartels & Zeki, 2004; Verhoeff *et al*., 2003) and the brain dopaminergic network, which projects to the prefrontal cortex from the midbrain region via hypothalamus, is known as the "brain reward system" (Martin-Soelch *et al*., 2001; Ikemoto, 2007). Previous studies have demonstrated that attraction for a favorite person activates brain reward system (Matsunaga *et al*., 2008b; Aron *et al*., 2005; Bartels & Zeki, 2004). The activation of brain reward system increases peripheral circulating dopamine level and dopamine may enhance NK cell activity through its dopamine receptors (Matsunaga *et al*., 2008b). NK cells can produce several cytokines such as interferon-gamma (IFN-γ) (Feng *et al*., 2006) and such cytokines released by lymphocytes during immune reactions can induce or upregulate the expression of MHC (Goes *et al*., 1995). The present study indicated that the positive correlation between the feeling of happiness and β_2-microglobulin; therefore, it is suggested that the positive emotion, couple's happiness, may be induced by dopaminergic system, and this system may stimulate NK cell activity and the expression of MHC. This study also indicated the negative correlation between the feeling of irritation and β_2-microglobulin. It may be because prolonged psychological stress is known to reduce the number of circulating lymphocytes and NK cell activity, thereby decreasing immune defense (Maisel *et al*., 1990). Furthermore, previous studies have also suggested that dopamine may increase serum albumin and fibrinogen levels (Christiansen *et al*., 1988; Abe *et al*., 2007). Based on these previous studies, it is possible that brain reward system, several hormones, and cytokines, such as dopamine and IFN-γ, may be associated with the increase of serum albumin, β_2-microglobulin, and fibrinogen levels.

MHC class I molecules are composed of two subunits, α-chain and β_2-microglobulin β-chain, and the α-chain is known to be highly polymorphic (Jacob *et al*., 2002; Garver-Apgar *et al*., 2006); there are many different variants individuals could possess at each gene site. Interestingly, recent studies have demonstrated the preferences for mates that possess genes dissimilar to one's own MHC (Jacob *et al*., 2002; Garver-Apgar *et al*., 2006). MHC sharing negatively predicts women's sexual responsivity to and sexual satisfaction with partners, suggesting that the MHC may be involved in romantic love. This preference may adaptively function to increase heterozygosity and thereby immunocompetence of offspring. The MHC is considered to be a source of unique individual odors and people can detect the odors encoded by genetic information (Jacob *et al*., 2002). The present study indicated that the serum level of β_2-microglobulin, an component of the MHC class I molecules, increased after warm partner contact and the feeling of happiness was positively correlated with the serum level of β_2-microglobulin. Based on present and previous studies, the MHC class I molecules may be a 'love protein' that may be associated with mate preference and couple's happiness.

Certain limitations of this study must be recognized. First, although we have already reported the effects of positive emotions on psychological and physiological indices in a small sample size (Matsunaga et al., 2008a; 2008b), the relatively small sample size (n = 16 samples) was insufficient to determine the psychological and physiological effects of warm partner contact. Thus, data from this experiment may be very preliminary. The generalizability of the present findings must be further tested using a larger sample size. Second, the participants in the present study have relatively-passionate love, which was indicated by PLS. However, because there is a kind in love, e.g. early stage passionate love or long term companionate love, the psychological and physiological responses may be different from the present data when elderly couples, which may have long term companionate love, do warm partner contact. Third, in the present study we could not analyze changes of small peptides and proteins in the serum, such as oxytocin (1007.19 Da) and ACTH (2578.93 Da), due to technical difficulties. Because a lot of peptides can influence psychological and physiological states in couples, for example oxytocin may be associated with love (Esch & Stefano 2005a; 2005b; Stefano & Esch 2005), it is highly possible that other peptides and proteins may be changed in the warm contact condition. Nevertheless, the present study indicated new insights of psychological and physiological effects of warm partner contact in healthy couples. The results may expand the scope of clinical literature that addresses the links between romantic love and health and well-being.

Acknowledgments

We thank Profs Masashi Yoneda and Kunio Kasugai (Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Aichi Medical University, Japan) for their suggestions and encouragement throughout this study. We also thank Dr. Minoru Fukayama (Central Research Laboratories, Aichi Medical University, Japan) for his excellent technical assistance. This work was supported in part by Grant-in-Aid for Scientific Research of the Japan Society for the Promotion of Science (16330136 to H.O.), and Health and Labour Sciences Research Grant on Research on Occupational Safety and Health from Japan Ministry of Health, Labour, and Welfare (H17-RODO-5 to H.O.). Masahiro Matsunaga has received a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientist (17007478).

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