

# Whey Protein Regulates of Some Surface Molecules Expressed on Monocytes: Significance for Amelioration of Some Diabetes Disorders

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## Abstract

**Objective:** Whey protein (WP) is an extract of a camel milk protein that could be played an important role in diabetic wounds. Diabetes mellitus has decreased wound healing due to their effects in leukocyte adhesion molecules, CD11b and CD18, and antigen presenting co-stimulatory molecules (CD80 and CD86). This work is aimed to evaluate the role of WP in the regulation of the CD11b, CD18, CD80 and CD86 appearance on monocytes.

**Methods:** In whole blood monocytes of control, diabetic and WP-treated diabetes rats were experienced for some surface molecules using ant-CD11b-FITC, ant-CD18-PE, ant-CD80-FITC and ant-CD18-PE for flow cytometry.

**Results:** Expression of CD11b, CD80 and CD86 on monocytes was increased significantly in diabetic animals after five weeks in association to the control subjects and the WP-treated diabetic animals ( $P < 0.01$ ). In contrast, the CD18 expression by monocytes of WP-treated diabetic group was improved significantly in relationship to diabetic group which was minor than the control animals ( $P < 0.01$ ).

**Conclusions:** The presented results showed that WP could be involved in down-regulation of the countenance of CD11b, CD80 and CD86, and the CD18 up-regulation on monocytes of diabetic animals. The significant finding of the investigation is the amelioration of some diabetes disorders. This study adds to the responsibility of monocytes in diabetes.

**Keywords:** Diabetes; Whey protein; Monocytes; CD11b; CD18; CD80; CD86

## Introduction

Diabetes is a syndrome detected by chronic hyperglycemia, vascular complications and abnormalities [1]. Proliferation of marginal blood monocytes was initiated by WP, camel milk [2]. Monocytes recruitment from the blood brook involves the appearance of adhesion molecules on the activated monocytes and vascular endothelium, as well as the transmigration and adhesion of the monocytes [3]. Then, monocytes will interrelate with extracellular matrix proteins and will discriminate into dendritic cells and macrophages. Some leukocytes alterations are finding in both Diabetes types [4]. Abnormalities of leukocyte role have been shown to occur during inflammation in diabetic rats [5], comprised leukocytes number decrease in inflammatory lesions and reduced production and transcription of pro-inflammatory cytokine (tumor necrosis factor (TNF)- $\alpha$ ) [6]. The dysfunction Lymphocyte might be the infections cause of higher frequency in diabetics, where a high quantity of apoptotic lymphocytes was found in diabetic animals and diabetic patients [7]. The metabolic disorder and therapeutic labors to restore metabolism of glucose may inspire part of the pragmatic changes in lymphocyte subsets in each diabetic type were proposed by some authors [8]. Elevated interleukin (IL)-8, a potent chemoattractant for T-lymphocytes and neutrophils, serum levels were created in type 1 and 2 diabetic animals, telltale that this cytokine might concern with the progress of diabetic macroangiopathy [9].

Some leukocytes attach firmly, wander through the endothelial surface, diapedese between endothelium, and after that travel through a subendothelial medium using endothelial receptors in a bond tumble [10]. CD18 ( $\beta 2$  integrin) expressed on leukocytes can pair with a number of other integrins (CD11) subunits that can join a multiplicity of legends, including the defy receptors fibrinogen, ICAM-1, -2, and -3; the complement fragment iC3b; and polysaccharides [11]. CD11/CD18

are adhesion receptors that encourage interface of leukocytes with each other [12], with endothelial cells during transmigration [13], and with specific opsonins deposited on invading bacteria and discarded or hypoxic tissues [14].

CD80 and CD86 participate in an important function in immune creation and immune responses of T-helper in a numeral of sickness models and alteration of their appearance can have deep effects on the expansion of immune responses [15]. There are few studies on the surface molecule expression on diabetic monocytes. Therefore, the intend of this work was to hypothesize that the CD11b and CD18 adhesion molecules and co-stimulatory antigen presenting molecules (CD80 and CD86) regulation in diabetic animals might be enhanced by their diets supplementing with separated protein fractions extracted from the protein of camel milk (WP).

## Methods

### Whey protein extraction

Using an IEC Model K centrifuge milk was skimmed at 5,000 g for 20 min (Boston, USA). Skim milk was acidified to pH 4.3 using 1M HCl.

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By centrifugation the precipitated casein was disinterested, while the WP in the supernatant was saturated with 70% saturated ammonium sulfate and incubated all over the night at 4°C. The precipitated WP was unruffled by centrifugation and dialyzed against distilled water at 4°C for 48 h using a Spectra/Pro® Membrane, MWCO 6,000-8,000 DA. The dialyzates obtained were lyophilized using a Uinitop 600SL (Virtis Company, Gardiner, NY 12525, USA) and were kept on -20°C until use. The un-denatured whey proteins in the dialyze were freeze-dried and cooled until use.

### Ethical approval

A Majaheem camel breed obtained from the area of Najd (Alazera farm; GPS: 300 02 47/ 300 02 27) in SKA was used to obtain camel milk. Accurate permissions were not necessary for activities in this farm. Scarce or sheltered species did not engage in this study. All experimental procedures involving animals were conducted in accordance to the Team for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health and approved by Animal principles group in the Zoology Department in the Faculty of Science at King Saud University.

### Experimental animals

Diabetes induction was by a single insertion of newly dissolved STZ (60 mg/kg of body weight; Sigma, USA) in a 0.1 Mol/l citrate buffer (pH 4.5) into the peritoneum [16]. Managed animals were injected with citrate buffer. After seven days of STZ insertion, the rats were experienced for levels of serum glucose. Rats with a serum glucose level  $\geq 200$  mg/dl after 2 h of glucose ingestion were considered diabetic and chosen for further studies.

Animals were appended with WP in the diet as heretofore described [17]. To prepare 500 gm of the diet, 25 gm mineral salts, 5 gm vitamins, 40 gm fats, 50 gm sucrose, 100 gm protein (20% protein) and 280 gm starch were mixed. In both the control and the diabetic groups, casein was the protein source. The un-denatured camel milk WP was the protein supply in the diabetic group treated with WP. The diet was reserved at 4°C until use [18]. Fifteen male rats (3-month-old), weighing 120-150 gm each, were getting from the Central Animal House of the Faculty of Pharmacy at King Saud University. All rats were put in metal cages inside a good airy room for 2 weeks before the experiment. Animals were under ordinary laboratory circumstances (relative humidity was 60%-70%, temperature at 23°C and a 12-h cycle of light/dark) and were nurtured a diet of standard trade pellets and assumed water ad libitum. Animals were classified into three investigational groups (n=5/group): group I was a control group, group II diabetic rat provided for five weeks with distilled water (250  $\mu$ l/rat/day) and group III was supplemented with WP (100 mg/kg/ body weight dissolved in 250  $\mu$ l/day) for five weeks.

### Whole blood flow cytometry

Whole blood samples were assorted with monoclonal antibodies [anti-CD11b-FITC, anti-CD18-PE, anti-CD80-FITC and anti-CD86-PE (Serotec; Oxford, UK)] and kept in a dark place for half an hour at room temperature. Erythrocytes were analyzed with FACs lysis buffer (BD; diluted into HBSS with 0.5% human serum albumin and 0.1% sodium azide). Samples were fixed by 1% paraformaldehyde. Immediately or within 24 hours of fixation, a FACScan Analyzer (BD Biosciences, San Jose, CA, USA) with software (BD) was used for flow cytometry that was standardized daily and cleaned vigilantly before each test acquisition. Mean fluorescence intensity (MFI) of CD11b, CD18, CD80 and CD86 were deliberate in the respective gates of monocytes.

### Statistical analysis

SPSS program version 19.0 was used for data analysis. The statistical analysis of the obtained data was performed using ANOVA test followed by least square differences (LSD) analysis for assessment between means. The results were articulated as mean  $\pm$  standard error (SE). Values of  $P < 0.01$  were measured statistically significant, while value of  $P > 0.01$  were painstaking statistically non-significant.

### Results

#### Glucose and insulin levels of the blood

The tested samples showed that the glucose level increased in the diabetic animals (279.13  $\pm$  29.923) than the control group (82.42  $\pm$  9.402) and the WP-treated diabetic animals (106.32  $\pm$  8.232). In contrast, the insulin level of the diabetic animals (5.11  $\pm$  2.402) was meaner than either the control group (13.81  $\pm$  3.501) or the WP-treated diabetic group (10.93  $\pm$  2.412). These data were confirmed statistically, where there were a very high significance among all groups ( $P < 0.001$ ).

#### CD11b expression on monocytes

After five weeks, the mean fluorescence intensity (MFI) of CD11b appearance in the monocytes respective gate of control group (52.64%) is shown in Figure 1A. In the diabetic group, it was increased recording 70.27% (Figure 1B) in the evaluation to the WP-treated diabetic group counting 55.46% (Figure 1C). Statistical analysis showed that the diabetic group (71.50  $\pm$  0.664) was significantly increased than both the control (51.59  $\pm$  0.631) and WP-treated diabetic group (54.49  $\pm$  0.503) ( $P < 0.01$ ).

#### CD18 expression on monocytes

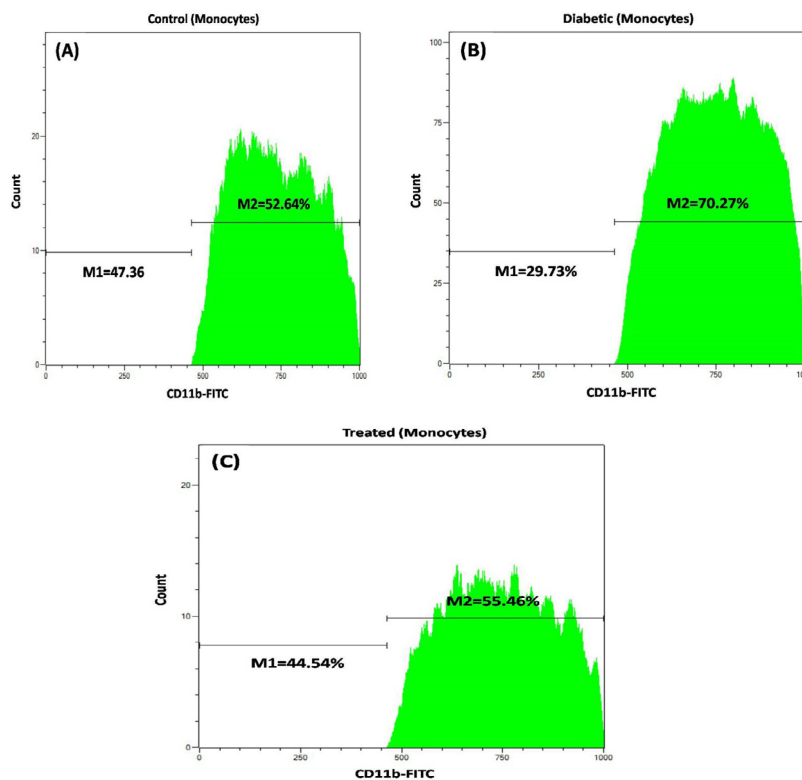
In the following set of experiments, we tested the MFI of CD18 appear in the respective gate of monocytes. Most monocytes (nearly 98%), tested by flow cytometry in diabetic animals after five weeks, showed a decrease in CD18 recording 42.90% (Figure 2A) with respect to the control group which showed a high amount (59.92%) of CD18 (Figure 2B). The WP-treated diabetic group was similar to the control counting 59.93% (Figure 2C). ANOVA statistical analysis of these data confirmed this result, where there is a significant difference between the control (60.76  $\pm$  0.477), diabetic (41.73  $\pm$  0.696) and WP-treated diabetic groups (60.72  $\pm$  0.546) ( $P < 0.01$ ).

#### Expression of CD80 on monocytes

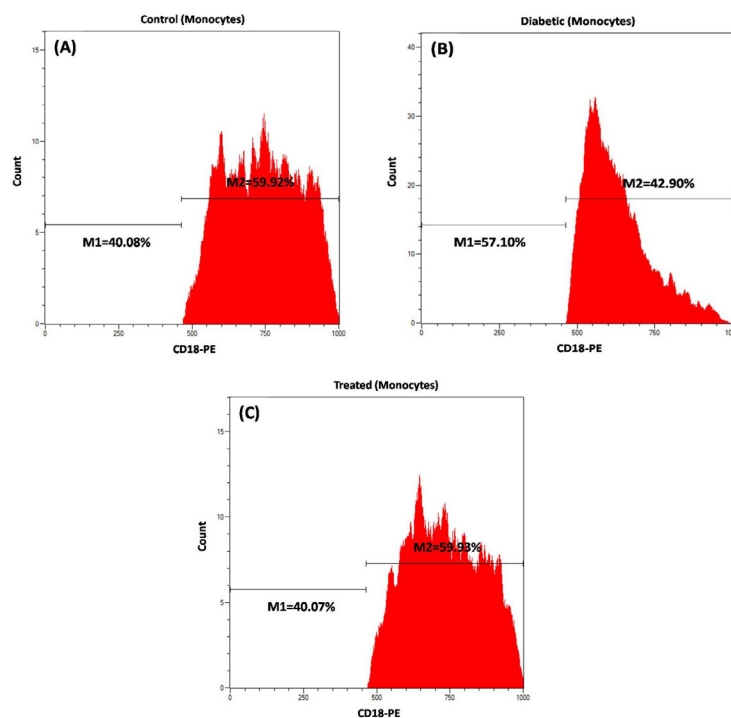
The MFI of CD80 expression in the respective gate of monocytes of the diabetic group, 27.92%, (Figure 3A) is higher than the control group, 15.13%, (Figure 3B) and the WP-treated diabetic group, 19.04%, (Figure 3C). Statistical analysis confirmed these results, where there are significance differences between all groups; control (16.14  $\pm$  0.630), diabetic (28.92  $\pm$  0.560) and treated (18.22  $\pm$  0.434) ( $P < 0.01$ ).

#### CD86 expression on monocytes

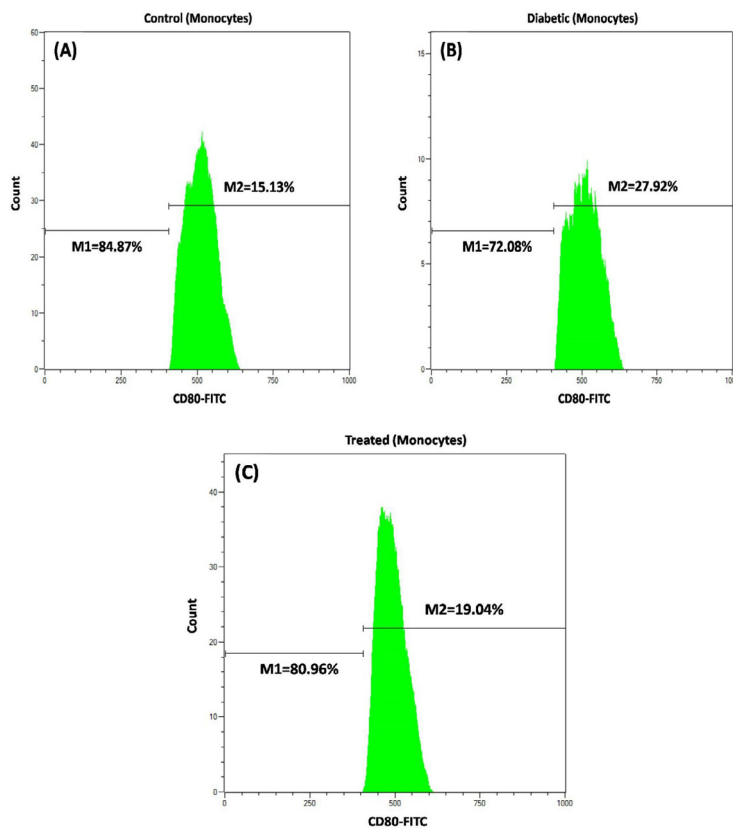
In this set of experiments, we experienced the MFI of CD86 expression in the individual gate of monocytes, where we found that the control group after five weeks counting 16.56% (Figure 4A) is nearly close to the WP-treated diabetic group (13.28%) (Figure 4B) and lower than the diabetic group (23.91%) (Figure 4C). Statistical analysis showed that there are significant differences between the control (15.03  $\pm$  0.837), the diabetic (24.89  $\pm$  0.568) and treated groups (14.61  $\pm$  0.726) ( $P < 0.01$ ).



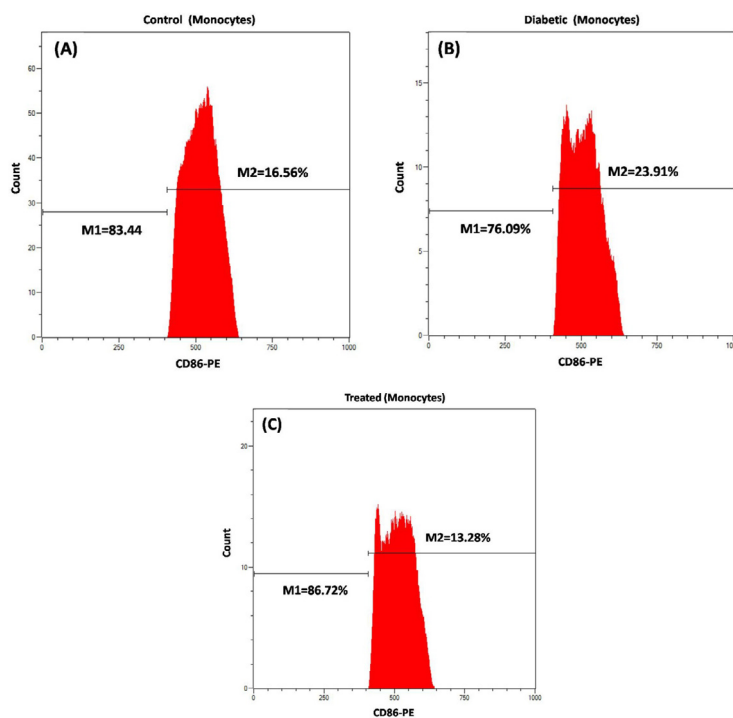
**Figure 1:** Direct fluorescence staining of whole blood with monoclonal anti-CD11b-FITC and flow cytometry measurements of the mean fluorescence intensity (MFI) in the gated monocytes after five weeks. A) Control group. B) Diabetic group. C) WP-treated diabetic group.



**Figure 2:** Direct fluorescence staining of whole blood with monoclonal anti-CD18-PE and flow cytometry measurements of the mean fluorescence intensity (MFI) in the gated monocytes after five weeks. A) Control group. B) Diabetic group. C) WP-treated diabetic group.



**Figure 3:** Direct fluorescence staining of whole blood with monoclonal anti-CD80-FITC and flow cytometry measurements of the mean fluorescence intensity (MFI) in the gated monocytes after five weeks. A) Control group. B) Diabetic group. C) WP-treated diabetic group.



**Figure 4:** Direct fluorescence staining of whole blood with monoclonal anti-CD86-PE and flow cytometry measurements of the mean fluorescence intensity (MFI) in the gated monocytes after five weeks. A) Control group. B) Diabetic group. C) WP-treated diabetic group.

## Discussion

The expression of CD11b and CD18 on monocytes in diabetic animals of the obtained data could be affected by different factors such as leukocyte proliferation and their cytokines. The up-regulation of monocytes CD11b of diabetic animals and their modulation in WP-treated diabetic animals of our results could be due to cell proliferation and/or cytokine production, where leukocytes creation results recommended that diabetic rats had an amplify in monocytes [19]. Circulating monocytes, in inflammatory condition, change from quiet, non-adhesive circumstances into an adhesive state characterized by a bigger appearance of adhesion molecules and an increase in well-organized adhesion [9]. Bahaa et al. also cited that a complete recapture of the impaired responses was realistic under the power of insulin [19]. Pro-inflammatory activated monocytes in the flow attach to the endothelium via an interface between adhesion molecules like Mac-1 (the heterodimer of CD11b and CD18) and the endothelial adhesion molecules approximating VCAM-1 and ICAM-1 [20].

Reinhold et al. indicated that high concentration of glucose significantly affected cytokine creation [21]. In accordance, Bahaa showed previously changes in the level of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-4 and IL-8 between the diabetic group and the other groups [19]. The changes in the level of TNF- $\alpha$  could be due to the changes in the discharge of insulin, where it modulates the growth of the inflammatory reply to allergen face by its ability to modulate the assembly/release of TNF- $\alpha$  [22]. ELISA serum analysis of level of TNF- $\alpha$  protein showed that it was elevated by diabetes. Nonetheless, RT-PCR examination of nearby wounded skin tissues shown that diabetes down-regulated the RNA expression of both TNF- $\alpha$  while, WP was found to significantly restore its RNA expression. The increased TNF- $\alpha$  level in the serum of diabetic wounds might be due to TNF- $\alpha$  complete effect of its pro-inflammatory deeds in diabetes [23], while its locally decreased RNA expression in diabetic wounds might be understandably responsible for the diminished staffing of inflammatory cells. Histological assessment here confirmed this clarification since the number of granulation cells and inflammatory cells were declined in diabetic wounds in evaluation to those of controls [24].

In addition, the down-regulation of CD18 on monocytes of diabetic animals and their modulation in WP-treated diabetic animals might be due to cytokine inhibition, where Reinhold et al. showed that elevated concentration of glucose significantly obstructs peripheral monocytes production by inhibiting the endogenous creation of IL-2, IL-6 and IL-10 [21].

The discrepancy appearance of co-stimulatory molecules on monocytes is thought to have fun a vital role in directing the answer of T-cell to pro-inflammatory or regulatory effectors' functions [25]. Preceding studies on antigen presentation in monocytes have exposed that the exogenous organization of CGRP impedes APC-driven T-cell proliferative replies by shifting CD86 appearance in APCs, a cause facilitated by the CGRP1 receptors [26]. In conclusion, the presented data here showed that WP involved in up-regulation of the CD11b, CD80 and CD86 expression and the down-regulation of the CD18 on monocytes of diabetic animals. These results could be accelerated the phagocytosis and antigen presentation processes of monocytes in diabetic patients with wounds.

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## Disclosure

The author declares that there are no conflicts of interest and relationships and affiliations.

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