Decreased mitogen response of splenic lymphocytes in obese Zucker rats is associated with the decreased expression of glucose transporter 1 (GLUT-1)\(^1\)\(^-\)\(^3\)

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**ABSTRACT** We reported previously that obesity is a risk factor for deteriorating cellular immune functions in aging. However, the mechanism by which obesity decreases cellular immunity remains to be elucidated. To determine the mechanism of the decrease in cellular immunity with obesity, lean (*Fa/fa*) and obese (*fa/fa*) 12-mo-old Zucker rats were used. The mitogen response of splenic lymphocytes in obese Zucker rats was significantly lower than that of lean Zucker rats, which was not restored by in vitro treatment with indomethacin (10 \(\mu\)mol/L), an inhibitor of prostaglandin E\(_2\) (PGE\(_2\)). In addition, PGE\(_2\) production by splenic lymphocytes was not greater in obese than in lean Zucker rats. Glucose consumption by splenic lymphocytes after in vitro incubation with concanavalin A (conA) for 48 h was also significantly lower in obese Zucker rats. Expression of glucose transporter 1 (GLUT-1), analyzed by Western blot analysis, was lower in splenic lymphocytes of obese than in lean Zucker rats. However, the expression of the conA receptor in splenic lymphocytes, analyzed by flow cytometry with fluorescein isothiocyanate–conjugated conA, was not significantly different between lean and obese Zucker rats. In conclusion, the decreased mitogen response of splenic lymphocytes in obese Zucker rats may be in part due to the decreased uptake of glucose as the main energy source for lymphocytes at the stage of proliferation and may be associated with the decreased expression of GLUT-1.


**KEY WORDS** Obesity, mitogen response, prostaglandin E\(_2\), glucose consumption, glucose transporter 1, concanavalin A receptor, Zucker rat, thymidine

**INTRODUCTION** Previous studies showed that obese subjects are more susceptible to cardiovascular disease, hypertension, cerebrovascular disease, and diabetes mellitus than are nonobese subjects (1). They have a higher incidence of infection and some types of cancer (2, 3), suggesting impaired immune function. In humans, only a few studies have directly compared specific immune responses in obese and nonobese subjects. It is known that obesity induces decreases in both T lymphocyte response to concanavalin A (conA) and B lymphocyte response to pokeweed mitogen (4). In addition, a negative correlation between percentage body fat and natural killer cell activity was found in both elderly women (5) and adult men (6). Elderly people (> 60 y of age) are also at risk from an increased incidence of infection. Their peripheral blood lymphocytes show an impaired proliferative capacity and a decreased reactivity to mitogens (7). We found and reported that obesity suppresses lymphocyte functions, natural killer cell activity, and lymphocyte mitogenesis in men and women > 60 y of age (8). This suggests that obesity is a risk factor for deteriorating cellular immune functions. However, the mechanism by which obesity decreases cellular immune functions remains to be elucidated.

Because Meydani et al (9) reported that decreased cellular immunity in elderly people was associated with increased production of prostaglandin E\(_2\) (PGE\(_2\)), the decrease of cellular immunity in obese elderly subjects may be induced by increased production of PGE\(_2\). We found that glucose is the main energy source for lymphocytes at the stage of proliferation (10). Decreased proliferation of peripheral blood lymphocytes in obese subjects may be related to impairment of glucose uptake by lymphocytes. It is known that glucose transport into cells is performed by glucose transporter (GLUT) expressed on their membranes (11). In particular, GLUT-1 is expressed on lymphocyte membranes and transports glucose into lymphocytes after mitogen stimulation. Obesity may modulate the expression of GLUT-1 in lymphocytes. Mitogen response is initiated by the binding of mitogen to mitogen receptors. Because T cell mitogens such as phytohemagglutinin and concanavalin A (conA) are present in sufficient amounts under in vitro incubation with splenic lymphocytes, a decreased mitogen response in obese subjects may be due to decreased expression of mitogen receptors on their lymphocytes.

Zucker rats increase their body weights by overfeeding, which induces hyperlipidemia and hypercholesterolemia (12). Although plasma insulin concentrations are several times higher in obese than in lean Zucker rats, plasma glucose concentrations are sim-
ilar (13). These observations suggest that although obese Zucker rats are not frankly diabetic, they are insulin resistant.

Therefore, we designed the present studies using Zucker rats as a model for obesity to investigate, first, whether a decreased mitogen response in obese rats is associated with increased production of PGE₂, second, whether decreased mitogen response in obese subjects is related to an impairment of glucose transport in lymphocytes and, third, whether obesity induces decreased expression of mitogen receptors on lymphocytes.

MATERIALS AND METHODS

Animals and diet

Zucker rats were originally obtained from Kiwa Experimental Animal Co, Ltd (Wakayama, Japan) and were propagated in our laboratory. In this experiment, 12-mo-old female Zucker rats were used. They were fed a nonpurified diet (MF; Oriental Yeast Co, Ltd, Tokyo) after weaning. At 8 wk of age they were divided into groups of 10 lean (Fa/?) and obese (fa/fa) rats. Until the end of the experiment (12 mo of age), all rats had free access to food and drinking water and were housed in a rodent facility at 22 ± 1 °C with a 12-h light-dark cycle. Body weight and food intake were measured daily. The study was conducted in accord with the Helsinki Declaration of 1975 as revised in 1983.

Blood collection and measurements of plasma glucose, insulin, and triacylglycerol

Blood was drawn from the inferior vena cava of each rat into syringes with heparin at the end of the experiment just before the animals were killed. Plasma obtained by centrifugation at 600 × g for 20 min at 4 °C was stored in aliquots at −40 °C until glucose, insulin, and triacylglycerol concentrations were determined. Concentrations of glucose, insulin, and triacylglycerol in plasma were measured by using a Glucose B-Wako test kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan), a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden), and a Triglyceride E-Wako test kit (Wako Pure Chemical Industries), respectively.

Preparation and mitogenesis of splenocytes

Rats were anesthetized with sodium pentobarbital (1 μL/g body wt) and exsanguinated by cutting off the arteries of both kidneys. The spleen was aseptically excised, weighed, and minced with scissors. The splenocytes were dissociated by using a stainless steel screen and adjusted to 1 × 10⁹ cells/L RPMI 1640 medium (Life Technologies, Inc, Grand Island, NY) supplemented with 25 mmol HEPES/L, 1.0 mmol L-glutamine/L, 100 mg streptomycin/L, and 50 mmol 2-mercaptoethanol/L. One hundred microliters of each cell suspension was cultured for 72 h at 37 °C with 100 μL conA (5.0 μg/L), and then the cultures were immediately pulsed with 37 kBq (1.0 μCi) [³H]thymidine. Twenty-four hours later, the splenocytes were harvested and their radioactivity was measured by using a liquid scintillation spectrometer.

Expression of conA receptors on splenic lymphocytes

Splenic lymphocytes were isolated from splenocytes by Percoll gradient centrifugation as reported previously (14). Isolated splenic lymphocytes (1 × 10⁶ cells/L) were cultured with fluorescein isothiocyanate–conjugated conA (5 mg/L; Vector Laboratories, Inc, Burlingame, CA) for 1 h at 37 °C with a 1:2500 dilution of rabbit anti-rat GLUT-1 antibody purchased from East-Acrea Biologicals (Southbridge, MA). The immunoblot was subsequently incubated for 1 h with a 1:2500 dilution of horseradish peroxidase–linked donkey anti-rabbit immunoglobulin antibody (Amersham, Buckinghamshire, United Kingdom). Positive bands were visualized by using the ECL detection system from Amersham. Expression of GLUT-1 in splenic lymphocytes was quantitated by using a laser densitometer (Ultrascan XL; Pharmacia LKB, Uppsala, Sweden).

Prostaglandin E₂ production by splenocytes

Splenocytes (1 × 10⁶ cells/L) were incubated with conA (5 mg/L) in a 5% CO₂ incubator at 37 °C for 48 h. Then the supernates of the splenocyte cultures were harvested and used for PGE₂ analysis. PGE₂ concentration in the supernate was determined by using the prostaglandin E₂ enzyme immunoassay system (Amersham International plc, Amersham, United Kingdom).

In vitro treatment with indomethacin

The effect of indomethacin on the mitogen response of splenocytes was measured as follows: 10 μmol indomethacin/L was added to the culture of splenocytes with conA (5 mg/L), which was incubated for 72 h at 37 °C and then pulsed with 37 MBq (1.0 μCi) [³H]thymidine. Twenty-four hours later, the splenocytes were harvested and their radioactivity was measured by using a liquid scintillation spectrometer.
RESULTS

Body and spleen weights and number of splenocytes

Obese Zucker rats were hyperphagic compared with lean Zucker rats during the experiment, which resulted in significantly higher body weights (Table 1). The mean body weight of the obese Zucker rats at the end of experiment was about three times higher than that of the lean Zucker rats. On the contrary, there were no significant differences in spleen weight per gram body weight or in the number of splenocytes per gram spleen between lean and obese Zucker rats (1.45 ± 0.42 g compared with 0.98 ± 0.26 g, and 7.90 ± 1.09 × 10⁶ compared with 6.54 ± 0.85 × 10⁶ cells, respectively).

Plasma glucose, insulin, and triacylglycerol concentrations

Plasma insulin and triacylglycerol concentrations in the obese Zucker rats were significantly higher than those of the lean Zucker rats. However, there was no significant difference in plasma glucose concentration between obese and lean Zucker rats (Table 1).

Mitogen response and PGE₂ production in splenic lymphocytes

In this experiment, mitogenesis of splenic lymphocytes with conA was measured by using the incorporation of [³H]thymidine. As shown in Figure 1, mitogen responses of splenic lymphocytes from the obese Zucker rats were significantly lower at all concentrations of conA compared with those of the lean Zucker rats. However, there was little difference between the obese and lean Zucker rats in the optimum concentration of conA needed to induce maximum proliferation of splenic lymphocytes. Production of PGE₂ by conA-stimulated splenocytes was significantly lower in the obese than in the lean Zucker rats (Figure 2). Furthermore, the addition of indomethacin (10 μmol/L) to the cultures of splenic lymphocytes with conA (5 mg/L) had no effect on the decreased mitogen response in the obese Zucker rats (Figure 2).

Glucose uptake by splenocytes

Glucose uptake by splenocytes was measured after in vitro incubation with conA (5 mg/L) for 48 h. As shown in Figure 3, glucose uptake by splenocytes from obese Zucker rats was significantly lower than that of splenocytes from lean Zucker rats (P < 0.05).

GLUT-1 expression in splenic lymphocytes

It is known that the main glucose transporter expressed in immune cells such as lymphocytes and macrophages is GLUT-1. In this experiment, the expression of GLUT-1 in splenic lymphocytes after in vitro incubation with conA (5 mg/L) for 48 h was measured by using Western blot analysis. As shown in Figure 4, the expression of GLUT-1 in splenic lymphocytes of the obese Zucker rats was lower than that of the lean Zucker rats.

Expression of conA receptors in splenic lymphocytes

In this experiment, the expression of conA receptors in splenocytes isolated from lean or obese Zucker rats was measured by using fluorescein isothiocyanate–conjugated conA. As shown in Figure 5, there were no significant differences in the expression of conA receptors on splenic lymphocytes from lean and obese Zucker rats.

DISCUSSION

Currently, it is generally accepted that obesity is linked to the occurrence of hyperlipidemia, diabetes mellitus, atherosclerosis, infections, and some types of cancer (15). Some of these diseases appear to be associated with the decrease in cellular immunity in obese persons. Although there are several reports showing that obesity induces the decrease of cellular immunity (4, 16), the mechanism remains to be elucidated. Zucker rats were used in this study as a model for obesity. Although plasma insulin and triacylglycerol concentrations were much higher in the obese Zucker rats than in the lean Zucker rats (465% for insulin and 1151% for triacylglycerol), plasma glucose concentration was not different. Despite hyperinsulinemia and hyperlipidemia, obese Zucker rats do not have diabetes mellitus. Because diabetes mellitus has a major effect on the immune system (17), Zucker rats are a suitable model to investigate the effect of obesity on cellular immunity.

As shown in Figure 1, proliferation of splenic lymphocytes with conA was significantly lower in the obese than in the lean
Zucker rats. Because the optimum concentration to induce maximum proliferation of splenic lymphocytes was not very different between the lean and obese Zucker rats, the decreased proliferation of splenic lymphocytes from obese Zucker rats appears to be due to lower responsiveness rather than to insufficient stimulation with conA. Because there are some reports showing that the quality and quantity of dietary lipids modulate cellular immunity (18, 19) and that an increased plasma insulin concentration is associated with a decrease in cellular immune functions such as natural killer cell activity and proliferation of peripheral blood lymphocytes (20, 21), the lower responsiveness of splenic lymphocytes from obese Zucker rats may be, in part, related to the increased insulin and triacylglycerol concentrations in plasma.

Because the decrease in cellular immunity after a high-fat or high-polyunsaturated-fatty-acid diet is associated with increased production of PGE2 (22), the decreased lymphocyte proliferation of splenocytes from obese Zucker rats appears to be due to lower responsiveness rather than to insufficient stimulation with conA. Because there are some reports showing that the quality and quantity of dietary lipids modulate cellular immunity (18, 19) and that an increased plasma insulin concentration is associated with a decrease in cellular immune functions such as natural killer cell activity and proliferation of peripheral blood lymphocytes (20, 21), the lower responsiveness of splenic lymphocytes from obese Zucker rats may be, in part, related to the increased insulin and triacylglycerol concentrations in plasma.

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Obese Zucker rats have elevated plasma insulin and corticosterone concentrations relative to their lean littermates (30). Because corticosterone depresses immune functions (31), the increased corticosterone concentration in obese Zucker rats may also be associated with decreased cellular immunity.

We reported previously that the main energy source for mature lymphocytes is glucose, especially at the stage of proliferation after in vitro stimulation with mitogens (11). On the basis of this report, we postulated that the decreased lymphocyte pro-
phocytes of obese Zucker rats may be associated with the impair-
ment of glucose uptake by lymphocytes. Muscle glucose uptake is lower in obese than in lean Zucker rats, which is involved in reduced plasma membrane GLUT-4 protein content and a defect in the insulin-stimulated activation of GLUT-4 (32, 33). However, there are no reports on glucose uptake of lymphocytes in obese Zucker rats. We found that glucose uptake by splenic lymphocytes was significantly lower in obese than in lean Zucker rats. This suggests that obese Zucker rats have an impaired glucose transport system in their splenic lymphocytes. Although several glucose transporters have been found in various tissues, GLUT-4, a major glucose transporter, is expressed in muscle and fat tissues and is closely associated with diabetes mellitus (34). In immune cells, GLUT-1, but not GLUT-4, is expressed on the membranes after mitogen stimulation (35), which is not dependent on insulin stimulation (36). Expression of GLUT-1 was remarkably depressed in splenic lymphocytes of the obese compared with the lean Zucker rats in the present study. This suggests that decreased proliferation of splenic lymphocytes in obese Zucker rats is associated with the impairment of glucose uptake, which is due to the decreased expression of GLUT-1.

Because GLUT-1 expression is decreased in diabetes induced by streptozotocin (37) or alloxan (38), hyperinsulinemia may down-regulate the expression of GLUT-1. GLUT-1 expression depends on mitogen stimulation and signal transduction after mitogen stimulation. If the number of mitogen receptors is lower on cells from obese Zucker rats, it might result in the decreased expression of GLUT-1 on splenic lymphocyte membranes. However, there was no significant difference in the expression of mitogen receptors on splenic lymphocytes between the lean and obese Zucker rats (Figure 5). Thus, the decreased expression of GLUT-1 in splenic lymphocytes of obese Zucker rats is not associated with the number of mitogen receptors. GLUT-1 is highly synthesized by the activation of mitogen-activated protein kinase after mitogen stimulation or the stimulation of the mitogen-activated protein kinase cascade via protein kinase C activation by phorbol myristate acetate (39). Because glucose transport in heart muscle cells was depressed in obese Zucker rats, as was protein kinase C activity (40), decreased expression of GLUT-1 in splenic lymphocytes of obese Zucker rats may be associated with the impairment of signal transduction after mitogen stimulation.

In our previous study we found that obesity is a risk factor for deteriorating cellular immune functions with aging. In that study, the decreased cellular immunity in aged people (>60 y old) may have been due not only to the decreased responsiveness of immune cells but also to the decreased uptake of glucose through decreased expression of GLUT-1. In conclusion, this study gives experimental support for the hypothesis that the decreased cellular immunity, especially lymphocyte mitogenesis, in obese subjects is associated with the decreased uptake of glucose into immune cells, which is associated with the decreased expression of GLUT-1.

REFERENCES


![Figure 4](image-url)

**FIGURE 4.** Reduction of expression of glucose transporter 1 (GLUT-1) in splenic lymphocytes of lean and obese Zucker rats after concanavalin A (conA; 5 mg/L) treatment as shown by Western blots. Seventy-five micrograms protein from conA-stimulated splenic lymphocytes of lean or obese Zucker rats was subjected to immunoblots with anti-GLUT-1 antibody as described in Materials and Methods. n = 10.

![Figure 5](image-url)

**FIGURE 5.** Expression of concanavalin A (conA) receptors in splenic lymphocytes from lean or obese Zucker rats. After lymphocytes were isolated from spleens of lean and obese Zucker rats, they were immediately incubated with fluorescein isothiocyanate-conjugated conA (5 mg/L) for 1 h and then conA receptor expression was assessed by using a flow cytometer as described in Materials and Methods.


