INSULIN resistance associated with obesity, particularly visceral obesity, has been revealed to be involved in the pathogenesis of lifestyle-related diseases, such as type 2 diabetes, hypertension, non-alcoholic steatohepatitis, chronic kidney disease and arteriosclerosis. In addition, inflammation caused by chronic infections, such as periodontal disease, is known to cause deterioration in glucose tolerance and insulin sensitivity [1]. Chronic infections, such as periodontal disease, have been shown to induce increased circulating levels of interleukin (IL) -6, IL-1β, IL-21 and C reactive protein. Overproduction of these cytokines leads to reduced insulin sensitivity and deterioration of glucose tolerance. Moreover, a local inflammation can later give rise to a systemic inflammatory state. However, the effect of a chronic parasitic infection on glucose tolerance remains unclear. Parasitic helminth is known to secrete an immunosuppressive agent and anti-inflammatory materials to suppress an attack from a host [2]. Therefore, it is possible that a parasitic infection may provide the opposite effect to a bacterial infection regarding insulin sensitivity.

On the other hand, a chronic low grade inflammation in adipose tissue is currently considered as a main cause of insulin resistance associated with obesity. Monocyte chemoattractant protein-1 (MCP-1) over-expressing mice exhibit insulin resistance and macrophage infiltration in adipose tissue, whereas high fat diet-induced insulin resistance, inflammatory profile of adipose tissue and hepatic steatosis are prevented in MCP-1 deficient mice, as well as MCP-1 receptor deficient mice [3, 4]. These data indicate that MCP-1, which is secreted in hypertrophic adipocytes,
-induced macrophage accumulation in adipose tissue causes insulin resistance. Moreover, classically activated macrophage (M1 macrophage) expressing tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS) observed in obese adipose tissue have been shown to be more harmful than alternatively activated macrophage (M2 macrophage) expressing arginase 1 and IL-10 in lean adipose tissue [5].

In this study, we investigated the parasite, *Trichinella spiralis*, for its ability to effect glucose tolerance and affect the status of macrophage in adipose tissue. *T. spiralis* is a parasitic nematode which infects a wide variety of vertebrate hosts. After consumption of infected muscles, the larvae develop into adult worms in the host intestine where the females give rise to the second generation. The newborn larvae migrate through the blood vessels and invade striated muscle cells. *Trichinella* infection causes local and systemic changes in the host. The former includes muscle cell transformation to a nurse cell in the cyst [6], and the latter includes immunological responses which act in two ways: one is resisting the second challenge of *Trichinella* infection and the other is suppressing the immune attack against the already-existing *Trichinella* in the host muscle. In relation to this research interest, we have previously studied cloning the cytokine macrophage migration inhibitory factor (MIF) homologue from *Trichinella* [7]. Another line of our study demonstrated that *Trichinella* infection improves glucose tolerance in control and obese mice without affecting adiposity.

**Materials and Methods**

1. **Animals and infection**

Ob/ob mice and C57/BL mice were fed CE2 powder (CLEA Japan Inc, Tokyo) *ad libitum*. In addition, C57/BL mice were fed a high fat diet (High Fat Diet 32, CLEA Japan Inc., Fat: 31.9%) for four weeks as high fat diet-induced obese mice (HF mice). They were divided into two groups (10 mice each): infected orally with 400 *Trichinella* larvae/mouse (infected group) and a control group (uninfected group) (Fig. 1). Four weeks later, body weight, fat weight (subcutaneous fat, epididymal fat and perirenal fat), fasting plasma glucose and insulin levels were measured as follows. To evaluate glucose tolerance, intraperitoneal glucose tolerance test (ipGTT, glucose: 2g/kg) was performed. Plasma glucose levels were measured with Medisafe Mini Blood Glucose Monitoring System (TERUMO, Tokyo, Japan), and plasma insulin levels were assayed with Insulin RIA kit.

![Fig. 1](image-url) Procedure for preparation of mice infected with (infected group) or without (uninfected group) *Trichinella*. C57/BL and ob/ob mice were fed with normal diet or high fat diet for 4 weeks. They were divided into two groups (10 mice each): infected orally with 400 *Trichinella* larvae/mouse (infected group) and uninfected group (control group). 4 weeks or 12 weeks later, experiments described in Materials and Methods were performed.
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(Tlinco Research, St. Charles, MO, USA). In addition, intraperitoneal insulin tolerance test (ipITT, insulin: 0.3 IU/kg) was performed in ob/ob mice. An area under the curve (AUC) was measured from glucose levels at 0, 30, 60 and 120 min during each ipGTT.

3. Immunohistochemical staining

Immunostaining of paraffin sections of the fat tissues was performed using anti-CD11c antibody and anti-CD206 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA).

4. Statistics

Statistical comparisons were performed with Student’s t-test (Fig. 2, Fig. 3C, Fig. 3F, Fig. 4C, Fig. 5 and Fig. 6). Other data were analyzed with one-factor ANOVA (Dunnett test). Data are given as mean ± SEM. Values of $P < 0.05$ were considered statistically significant.

Results

1. Effects of Trichinella infection on fasting plasma glucose level and glucose tolerance

Trichinella infection for four weeks reduced fasting plasma glucose level without influence on plasma insulin levels in C57/BL and ob/ob mice (Fig. 2A, B). These results implied that Trichinella infection improved insulin sensitivity. Trichinella infection did not affect body weight or fat weight (Fig. 2C-E). To evaluate the effect of Trichinella infection on glucose tolerance, ipGTT was performed. As shown in Fig. 3A-C, Trichinella infection for four weeks suppressed plasma glucose levels during GTT in ob/ob mice, but not in lean control mice as shown in Fig. 3A. It also improved glucose tolerance in high fat fed mice (Fig. 3E). On the other hand, ipITT performed in ob/ob mice revealed that insulin sensitivity was improved in the infected group compared with the uninfected group (Fig. 3G). Taken together, Trichinella infection improved glucose tolerance and insulin sensitivity without weight reduction especially in obese mice.

2. Immunocytotoxic staining

As Trichinella infection decreased plasma glucose level more effectively in obese animals, we speculated that it may act on adipose tissue inflammation. To observe infiltration of inflammatory cells in adipose
Fig. 2 (A) Fasting plasma glucose level in C57/BL: control (C57/C), C57/BL: *Trichinella* infected (C57/T), ob/ob: uninfected control (ob/C) and ob/ob: *Trichinella* infected (ob/T). *: p<0.05, vs C57/C (n=5). #: p<0.05, vs ob/C (n=10). (B) Fasting plasma insulin level in C57/BL and ob/ob. (C) Body weight in C57/BL and ob/ob. (D) Fat weight in C57/BL. White bar: uninfected control, black bar: *Trichinella* infected, Sub: subcutaneous fat, Epi: epididymal fat, Ren: perirenal fat, n=5, (E) Fat weight in ob/ob. White bar: uninfected control, black bar: *Trichinella* infected, Sub: subcutaneous fat, Epi: epididymal fat, Ren: perirenal fat, n=10. All data was measured 4w after infection.

Fig. 3 (A) ipGTT in C57/BL 4weeks after infection. (B) ipGTT in ob/ob 4weeks after infection. *: p<0.05, uninfected vs infected (n=10). (C) AUC of ipGTT shown in (A) and (B). *: p<0.05, uninfected vs infected (n=10). (D) ipGTT in high fat fed mice 4weeks after infection. *: p<0.05, uninfected vs infected (n=10). (E) ipGTT in high fat fed mice 12weeks after infection. *: p<0.05, uninfected vs infected (n=10). (F) AUC of ipGTT shown in (D) and (E). *: p<0.05, uninfected vs infected in 4weeks (n=10), #: p<0.05, uninfected vs infected in 12weeks (n=10). (G) ipITT in ob/ob 4weeks after infection. *: p<0.05, uninfected vs infected in 4weeks (n=6). Open square/broken line: uninfected, closed triangle/solid line: infected. White bar: uninfected, black bar: infected.
tissue isolated from ob/ob mice, we performed immunocytochemical staining in epididymal adipose tissue. The amount of CD11c-labeled cells decreased (Fig. 4A), whereas the amount of CD206-labeled cells increased in the infected group compared with the uninfected one (Fig. 4B). Crown-like structures (CLS), which represent dead adipocytes [8], were prevalent in adipose tissue isolated from uninfected ob/ob mice compared with infected ones (Fig. 4C).

3. RT-PCR

The expression levels of CD68 in adipose tissue isolated from infected ob/ob mice were smaller than those from uninfected ob/ob mice. When mRNA levels were normalized by G3PDH, the expression levels of M1 macrophage markers including CD11c, NOS2 and IL-6 decreased in adipose tissue isolated from the infected group, while M2 macrophage markers including CD206, arginase 1 and IL-10 increased (Fig. 5A) compared with the uninfected group. These results indicated that the amount of whole macrophage in adipose tissue decreased in the infected group, which was accompanied with a shift of macrophage phenotype from M1 to M2. Expression levels of mRNA for M1 and M2 markers, which was normalized by CD68, yielded similar results (Fig. 5B). Conversely, the expression levels of adipocyte specific genes including PPARγ, adiponectin, MCP-1 and leptin were not influenced by the *Trichinella* infection (Fig. 5A). We quantified the expression level of M1 marker and M2 marker in SVF isolated from epididymal fat in infected and uninfected ob/ob mice. Furthermore, to evaluate the effect of the

![Fig. 4](image-url)

(A) Representative immunohistochemical staining for CD11c (brown). Left: uninfected ob/ob mice, right: infected ob/ob mice. (B) Representative immunohistochemical staining for CD206 (blue). Left: uninfected control ob/ob mice, right: infected ob/ob mice. (C) Representative crown-like structure (CLS) (left) and mean prevalence of CLS per field (50 fields) (left). NC: Negative control. All scales represent 50 μm. *: p<0.05, vs Control.
Trichinella infection in a systemic macrophage fraction, we also measured M1 and M2 marker mRNA levels in residential macrophages in peritoneal lavage. The expression levels of M1 marker, CD11c and NOS2, were suppressed in peritoneal lavage (PT) and SVF, and IL-6 in PT isolated from infected mice. In contrast, the expression levels of M2 marker, CD206, arginase 1 and IL-10, were elevated in SVF and PT isolated from infected mice (Fig. 6). These results indicated that the Trichinella infection systemically shifted macrophage polarization from M1 to M2 in SVF and PT.

Discussion

Obesity leads to lifestyle-related diseases such as type 2 diabetes, hypertension, dyslipidemia, non-alcoholic steatohepatitis, chronic kidney disease, and atherosclerosis. Recently, it has been shown that a chronic low-grade inflammation of adipose tissue in obese men and animals is a main pathogenesis of insulin resistance, which gives rise to these metabolic diseases [5, 9]. Enlarged adipocytes produce excess fatty acid and inflammatory cytokines, such as MCP-1 and TNF-α. These changes...
in the adipose tissue microenvironment promote recruitment of macrophages and shift macrophage polarization. M2 macrophages, which are induced by Th2 cytokines such as IL-4 and IL-13, and express arginase 1, mannose receptors and IL-10, an anti-inflammatory cytokine, are predominantly detected in lean adipose tissue. M2 macrophages link to suppress an excess inflammatory process to rescue > prevent tissue damage. Conversely, M1 macrophages, which are induced by Th1 cytokines such as interferon (IFN)-γ and bacterial products, lipopolysaccharide (LPS), express NOS2 to produce cytotoxic NO, and secrete TNF-α, IL-6, IL-1β, IL-12 and CCL2, and increase the volume and cytokinetic activities in obese adipose tissue [5, 9, 10]. Much evidence has been accumulated to demonstrate that the obesity-induced shift of M1/M2 polarization and resultant adipose tissue inflammation is essential to provoke insulin resistance. Mice overexpressing diacylglycerol acyltransferase 1 in both macrophages and adipocytes were more inclined to diet-induced obesity but were protected against M1 macrophage activation and insulin resistance [11].

In this study, we have examined the effect of a parasitic infection on glucose tolerance. Chronic infection caused by bacteria such as Helicobacter pylori, Chlamydia pneumoniae and Porphyromonas gingivalis are correlated with the onset of atherosclerosis presumably via an inflammatory response induced by LPS [12]. As the circulating levels of LPS, which is a potent stimulator of M1 macrophage differentiation and production of inflammatory cytokines, negatively regulate insulin sensitivity, a recent study shows that modulation of bacterial infection by antibiotics improves insulin signaling [13]. In contrast, a parasitic infection is expected to influence insulin sensitivity and glucose tolerance differently, since parasites modulate inflammation negatively to protect themselves from their hosts [2]. The Trichinella family has seven species, such as Trichinella britovi, T. murrelli, T. native, T. nelson, T. papuae, T. pseudospiralis, T. spiralis, and several types of unclassified genotype [14]. Trichinella

![Graph showing expression levels of M1 (upper) and M2 (lower) markers mRNA in SVF (shaded bar) and peritoneal lavage (hatched bar) isolated from uninfected and infected ob/ob mice. RT-PCR was performed to quantify mRNA levels. Copy numbers of each gene were normalized with CD68. All values represent percent of each control as 100%. *: p<0.05, vs SVF control (n=5), #: p<0.05, ##: p<0.01 vs peritoneal lavage control (n=5)]
*Trichinella spiralis* utilizes almost all carnivores and omnivores as hosts. *Trichinella spiralis* has a distinctive life cycle that is an intermediate host as well as a final host. If a host ingests *Trichinella* orally, they perform sexual reproduction in the small intestine, with adult females laying their eggs in the small intestine mucosa, and then the newborn larvae are transported throughout the whole body mainly through the bloodstream, form a cyst in striated muscle fibers, and wait for the opportunity to be predated to the next host.

*Trichinella* infection decreased fasting plasma glucose level without any changes in body weight, or fat weight as shown in Fig. 2. As blood glucose levels were more rapidly and markedly reduced by insulin injection, insulin sensitivity improved in the infected group. Improvement of glucose tolerance evaluated by ipGTT was observed in obese mice including ob/ob mice and high fat fed mice. Ingestion of *Trichinella* causes body weight loss due to enteritis in the first week. It is gradually restored following the movement of the worms to skeletal muscle. The enteritis-induced weight loss could not be recognized four weeks after infection. Since no difference was observed in body weight and fat weight between control and infected groups, the improvement of glucose tolerance in the infected group is not due to malnutrition caused by the parasitic infection.

We performed immunostaining for CD11c and CD206 in adipose tissue of ob/ob mice as shown in Fig. 4. A more abundant expression of CD206 was detected in the infected group than in the uninfected group. A crown-like structure was less frequently observed in adipose tissue isolated from the infected group. These results suggest that obesity-induced chronic inflammation may be suppressed by *Trichinella* infection. The quantitative analysis of gene expression by RT-PCR showed that mRNA levels of CD68 was reduced in the infected group. Moreover, expression levels of M1 macrophage markers including CD11c, NOS2 and IL-6 were decreased, while M2 macrophage markers including CD206, arginase 1, IL-10 increased in whole adipose tissue, SVF and peritoneal lavage cells of residential macrophage isolated from the infected group as shown in Fig. 5, 6. These results indicated that improvement of glucose tolerance in the infected group was associated with reduced infiltration of macrophage and the shift of macrophage polarization toward anti-inflammatory phenotype in adipose tissue. Because residential macrophage in peritoneal lavage exhibited similar changes to macrophage in SVF, it is suggested that a parasitic infection may influence systemic immune function. As mesenteric fat links to systemic glucose and lipid metabolism more closely than epididymal fat [15], macrophage polarization in mesenteric fat was hoped for result and could be estimated. However, the contamination of small lymph nodes located in the mesentery interfered with the measurement of macrophage markers in SVF, making our method inappropriate to assess macrophage polarization in mesenteric fat.

No difference was observed in gene expression levels of adipocyte specific genes including PPARγ, adiponectin, leptin and MCP-1, in total RNA isolated from epididymal fat, between infected and uninfected groups as shown in Fig. 5. These results suggest that improved insulin sensitivity in the infected group may be due to the changes of adipocyte specific genes. Our results have been consistent with recent data suggesting that a shift of macrophage polarization toward M2 is sufficient to protect mice against diet-induced insulin resistance [11]. Although the expression of adiponectin, an insulin-sensitizing adipokine, was not influenced, another mechanism may be involved in *Trichinella* infection-induced improvement of insulin resistance. Since circulating IL-10 enhances insulin sensitivity *in vivo* and *in vitro* [16, 17], increasing the expression level of IL-10 in adipose tissue and peritoneal lavage (Fig. 5 and 6) may contribute to prevent insulin resistance. In this study, we examined the effects of *Trichinella* infection on inflammation of adipose tissue, however, the possibility cannot be excluded that other organs, such as the liver and skeletal muscle may be involved.

We have cloned the immunosuppressive agent in the excretory-secretory (ES) products from *Trichinella* [7]. Other researchers have demonstrated that a parasite such as *Trichinella spiralis*, *Schistosoma mansoni*, *Theromyzon tessulatum* and *Filaria* secretes substances that modulate immune response [18, 19]. Our preliminary experiments showed that treatment with ES decreased IL-6 mRNA level and increased IL-10 mRNA level in cultured macrophages (RAW264.7) (data not shown). Taken together, we hypothesize that secreted substances by *Trichinella* may induce the changes of systemic macrophage polarization followed by increased M2/M1 ratio in SVF, which may result in improved insulin sensitivity and glucose tolerance in obese mice. Identification of a specific substance may lead to a new strategy to treat type 2 diabetes and other inflammatory diseases.
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References


