Short chain fatty acids may elicit an innate immune response from preadipocytes: A potential link between bacterial infection and inflammatory diseases.

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The supporting laboratory work was funded by James Cook University’s School of Medicine.
Short chain fatty acids (SCFAs) such as acetate, propionate and butyrate are produced by bacterial fermentation of dietary fiber. The highest concentrations of SCFAs in the body are found in the colon. Elevated dietary acetate has been shown to have anti-inflammatory effects in mouse models of colitis and inflammatory diseases in peripheral tissues. The details of how dietary SCFAs stimulate reduced inflammation in peripheral tissues have not been determined. I suggest that SCFA concentrations in peripheral tissues are generally not sufficient to locally produce a significant anti-inflammatory effect from immune cells. Moreover it is possible that elevated SCFA levels in peripheral tissues may actually stimulate an inflammatory response. The hypothesis is presented that preadipocytes and other cells with immune function such as fibroblasts in peripheral tissues elicit an inflammatory innate immune response when exposed to SCFAs at mM concentrations. A role for SCFAs in activating an immune response in preadipocytes is possible given the expression of a SCFA receptor in these cells, the demonstration that adipocytes and preadipocytes have immunity related functions, the observation that 2 mM SCFAs stimulated the expression of monocyte chemoattractant protein-1 (MCP-1) mRNA from 3T3-L1 preadipocytes and that concentrations of SCFAs can reach elevated levels at sites of bacterial infection. A SCFA-induced inflammatory response from preadipocytes and other cells with immune function, such as fibroblasts, may provide a further contributing factor linking bacterial infection to the development of insulin resistance and the severity of inflammatory diseases such as atherosclerosis.
The highest levels of short chain fatty acids (SCFAs) such as acetate, propionate and butyrate are found in the colon and are produced by bacterial fermentation of organic matter (reviewed by 1). Two SCFA receptors, G protein-coupled receptors FFAR2 and FFAR3 (previously GPR43 and GPR41) have been described, and are activated by acetate, propionate, and butyrate (2). FFAR3 is predominantly expressed in adipose tissue and FFAR2 is predominantly expressed in immune cells (2).

There are reports that SCFAs reduce inflammation in inflammatory diseases acting through FFAR2. Maslowski et al. (3) demonstrated that colitis induced mice, fed 200 mM acetate, had reduced inflammation acting through the stimulation of FFAR2 on immune cells. The anti-inflammatory effect of dietary acetate was also observed in peripheral tissue in the K/BxN serum-induced mouse model of inflammatory arthritis and in the ovalbumin-induced mouse model of allergic airway inflammation (3). These authors did not indicate whether the local elevation of acetate produced the anti-inflammatory effect in peripheral tissues or whether it was an indirect effect due to elevated SCFA levels in the vicinity of the gastrointestinal (GI) system. They did not measure circulating acetate levels in their in vivo experiments.

Cox et al. (4) found that SCFAs at mM concentrations were needed to induce an anti-inflammatory in vitro response from human monocytes and peripheral blood mononuclear cells (PBMC) through the regulation of prostaglandin E\(_2\) (PGE\(_2\)), and cytokine and chemokine expression. This result was in spite of these author’s findings that acetate stimulated a calcium flux in human neutrophils with an EC50 of approximately 0.06 mM.
and a measured EC50 for acetate acting on FFAR2 of approximately 0.3 mM as obtained by Le Poul et al. (5).

I argue that the feeding of 200 mM acetate or the production of SCFAs in the GI system after ingestion of a natural diet are unlikely to elevate SCFA levels in peripheral tissues to mM concentrations and therefore any observed reduction in inflammation in peripheral tissue would not be due to the local stimulation of leukocytes. It is therefore possible that the elevation of SCFAs to mM concentrations in peripheral tissues may actually be inflammatory.

CONCENTRATIONS OF SHORT CHAIN FATTY ACIDS IN THE BODY

SCFA concentrations in the human colon usually range from 20 to 140 mM (reviewed by 1). Acetate represents approximately 60% of the SCFA produced in the colon and most of the remainder consists of similar proportions of propionate and butyrate. The majority of the SCFAs produced are absorbed by the caecum and the colon where they are used as an energy source by colonocytes. A significant proportion of acetate and most of the remaining butyrate and propionate are removed from the portal venous system by the liver. Concentrations of propionate and butyrate in peripheral circulation are generally low, ranging from 3 to 7 \( \mu \text{M} \) for propionate, and 1 to 4 \( \mu \text{M} \) for butyrate (6).

Levels of acetate are higher in peripheral circulation ranging from 70 to 170 \( \mu \text{M} \) (6) and can increase to mM concentrations after alcohol consumption (7-8). The regular consumption of alcohol, in sufficient quantities to elevate circulating SCFA levels to mM concentrations, would not be considered part of a natural diet from an evolutionary point of view (i.e. organ
and cellular systems were developed in pre-human times well before the organized production of alcoholic beverages).

In general, circulating levels of SCFAs are low, particularly for propionate and butyrate. In most cases SCFA in peripheral circulation are present in µM concentrations which were not sufficient to stimulate an anti-inflammatory response from immune cells (4). However, many pathogenic bacteria are capable of producing SCFAs and levels can be elevated to mM concentrations at sites of bacterial infection (9, reviewed by 10). The association between bacterial infection and elevated levels of SCFAs may have stimulated the evolution of a SCFA-induced (at mM concentrations) inflammatory response in peripheral tissues from cells with immune function.

SHORT CHAIN FATTY ACIDS AND INFLAMMATION

Sina et al. (11) found increased mortality of FFAR2 negative mice compared with controls in an acute colitis model due to increased bacterial translocation and sepsis. They concluded that there was a critical role for the recruitment of polymorphonuclear leukocytes (PMN) mediated by FFAR2 in both the containment of bacterial translocation and in chronic inflammation. Le Poul et al. (5) had previously suggested that FFAR2 has a function in recruiting leukocytes to sites of bacterial infection with concentrations of 1 mM having optimal effects on the chemotaxis of polymorphonuclear cells. An inflammatory role for SCFAs was also identified by Vinolo et al. (10); they found that SCFAs induced neutrophil migration and the exacerbation of inflammation in mice. SCFAs are therefore likely to have a role in innate immunity and the control of bacterial infections.
During experiments performed under the supervision and direction of Professor R.L. Kennedy, Obesity and Diabetes Research Group, School of Medicine, James Cook University, Townsville, Australia, (12) it was observed that SCFAs, particularly butyrate, at 2 mM concentration, stimulated the increased in vitro expression of monocyte chemoattractant protein-1 (MCP-1) (mRNA) from mouse 3T3-L1 preadipocytes, but not adipocytes, compared with negative controls. The stimulation of MCP-1 expression by SCFAs potentially represents an inflammatory immune response.

I present the hypothesis that SCFAs at mM concentrations in peripheral tissue stimulate an inflammatory response from preadipocytes, and possibly other cells with immunity related function such as fibroblasts, providing a further mechanism whereby bacteria potentially worsen inflammatory diseases.

Adipocytes play a role in immunity by influencing lipid metabolism and the production of adipokines but also through the expression of Toll-like receptors that respond to bacterial endotoxins and components of fungal cell walls (13). Chung et al. (14) found that endotoxin lipopolysaccharide (LPS) stimulated the production of inflammatory cytokines, including TNF-α, IL-6, and the chemokine MCP-1, from human preadipocytes. A more significant role in the immune response is demonstrated by the phagocytic and antimicrobial activity of preadipocytes and their ability to differentiate into macrophages (15).
A role for SCFAs in activating an immune response in preadipocytes is possible given the expression of FFAR3 in these cells (12), the demonstration that adipocytes and preadipocytes have immunity related functions, the observation of increased expression of MCP-1 from 3T3-L1 preadipocytes with exposure to 2 mM SCFA particularly butyrate, and as an evolutionary stimulus, the production of SCFAs by pathogenic bacteria that can be elevated to mM concentrations at sites of infection.

In addition fatty acids, as produced by catabolism during infection, can stimulate inflammatory responses including the activation of Toll-like receptors in adipocytes (16). It is therefore not unreasonable to suggest that SCFAs, including those endogenously produced after alcohol consumption, stimulate an innate immune response from preadipocytes and other immune related cells such as fibroblasts in peripheral tissue. Fibroblasts express MCP-1 and can produce an immune response, such as the expression of CD40 which is an immune cell activator (17).

**IMPLICATIONS: INFLAMMATORY DISEASES**

Obesity produces a low grade inflammation and is the major risk factor for developing insulin resistance and type II diabetes. Macrophages play a significant role in the development of insulin resistance and type II diabetes, through the production of inflammatory molecules such as TNF-α, IL-6, and MCP-1 (reviewed by 18). The importance of macrophages in the development of type II diabetes is suggested by the correlation of body mass index with macrophage density in adipose tissue and plasma concentrations of MCP-1 (18).
An inflammatory response from preadipocytes with exposure to SCFAs from infecting bacteria may further elevate MCP-1 and the infiltration of macrophages into adipose tissue in obese people, facilitating the progression to insulin resistance and type II diabetes. In addition bacterial lipopolysaccharides (LPS) can activate Toll-like receptors in human abdominal subcutaneous adipocytes to produce inflammation and induce an innate immune response (19). LPS activated preadipocytes induce insulin resistance in human adipocytes (14) and diabetes is known to worsen during infection (13).

Creely et al. (19) found that circulating bacterial endotoxins were elevated in type II diabetic patients and suggested that they may have originated from the gut. However, bacterial infections in adipose tissue, such as mycoplasma infected endothelial cells or even the mycoplasma infected macrophages may elevate LPS and SCFA concentrations to promote the development of type II diabetes.

Although controversial there is evidence linking bacterial infections to inflammatory and autoimmune diseases and the production of SCFAs may in some way contribute to disease intensity. *Mycoplasma pneumoniae* has been linked to atherosclerosis (reviewed by 20). The local production of acetate from this pathogen and the subsequent modification of cytokine and chemokine expression may contribute to the development and severity of atherosclerosis.

**FURTHER INVESTIGATIONS**

Experiments need to be performed with human cells including preadipocytes from different fat depots, and fibroblasts, to characterize the chemokines and cytokines that are expressed
due to SCFA signaling.

The effect of SCFAs on visceral preadipocytes will be of interest. Visceral fat is exposed to higher levels of SCFAs in the portal circulation, which, as indicated by Xiong et al. (21), can reach 0.45 mM. The proportions of different SCFA in the portal vein would also be more affected by diet than in peripheral circulation. In addition, visceral fat has an association with lymph nodes. Adipocytes in close association with lymph nodes support immune responses and prolonged low-level immune stimulation promotes adipocyte proliferation, especially near inflamed lymph nodes (22). Increased hypertrophy of visceral fat stimulated by low grade inflammation may explain the association of visceral adiposity, or obesity in general, with HIV, smoking, and various infections (as discussed by 22).

Preadipocytes in visceral fat are therefore likely to experience higher levels of SCFA, the quality of which will be influenced by diet to a greater degree than for subcutaneous preadipocytes, and an increased role in immunity.

Other cells from adipose tissue such as endothelial cells, pericytes, and leukocytes should also be investigated. Further in vivo studies should be considered to measure the effect of SCFA on leukocyte infiltration and macrophage density.

CONCLUSIONS

Short chain fatty acids (SCFAs) stimulate the expression of MCP-1 from 3T3-L1 preadipocytes. This is possibly an innate immune response. A further contributing factor linking bacterial infection to the development of insulin resistance and the severity of
inflammatory diseases has potentially been identified.

ACKNOWLEDGMENTS

Thanks to Dr Laura Grogan for her invaluable comments in reviewing the manuscript. The supporting laboratory work was funded by James Cook University’s School of Medicine. The author has no conflict of interest.

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