Modulation of immune function by medium-chain triglycerides

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Abstract

Triglycerides can be divided based on the length of their fatty acids. A distinction is made between short chain triglycerides (2-5 carbon atoms), medium chain triglycerides (6-12 carbon atoms) and long chain triglycerides (>12 carbon atoms). All are differently absorbed and metabolized in the body. Medium chain triglycerides are linked to many health benefits, including increasing satiety, decreasing energy consumption and weight loss. They are also indicated to reduce ageing of the brain and show anti-coagulation properties. Moreover, medium chain triglycerides are believed to influence immune responses. The aim of this thesis is to review the current literature on the effect of medium chain triglycerides on the immune system. It was found that medium chain triglycerides might be able to cause a disruption in tight junctions present in the intestinal epithelial barrier and also influence both pro- and anti-inflammatory cytokine secretion by cells in the intestine. Also influence of medium chain triglycerides on neutrophil infiltration in the intestine is described. However, data are inconsistent. Moreover, medium chain triglycerides seem to influence allergic responses. Both allergy stimulating and inhibiting responses after medium chain triglyceride supplementation are described. It can be concluded that little is known about the effect of medium chain triglycerides on immune cells and function. More research is needed.

Key words: *Triglycerides, MCTs, LCTs, immune responses, allergic reactions*

Summary

Triglycerides are a huge part of our diet. They are composed of a glycerol backbone with three fatty acids. These fatty acids exist of carbon atoms and differ in length. A distinction can be made between short chain triglycerides (SCT, 2-5 carbon atoms), medium chain triglycerides (MCT, 6-12 carbon atoms), and long chain triglycerides (LCT, >12 carbon atoms). Due to their difference in length, the fatty acids are differently absorbed and metabolized. Since MCTs are small they are easily absorbed and immediately used as energy source, whilst LCTs, which are difficult to absorb, are stored as fat. Since MCTs are directly used, it is suggested that an increase in MCT intake enhances satiety, causing a decrease in energy consumption resulting in a lowered body weight. Besides this, MCTs are also indicated to reduce ageing of the brain and have anti-coagulation properties. Recent work has demonstrated that fatty acids play a key role in immune responses to dietary antigens. The aim of this thesis is to look at the effect of MCTs on these immune responses, and to sort an list existing literature about this topic.

An influence of MCTs on tight junctions in the intestinal epithelium is found. Tight junctions are small openings between two cells. Research suggests that MCTs alter these tight junctions, causing a greater chance of antigens and pathogens entering the intestinal mucosa. Also cytokine and chemokine secretion is altered after administration of MCTs. Addition of MCTs in combination with a stimulus is more likely to result in an increased IL-8 secretion. Since IL-8 is responsible for neutrophil attraction, it is likely this leads to increased neutrophil infiltration. Neutrophils are small immune cells. An increase in these cells will result in an enhanced immune response. Moreover, presence of MCTs might lead to a decrease in NFκB activation, resulting in a lowering of pro-inflammatory cytokines and chemokines. MCTs are found to bind PPARγ or GPR84, which might lead to secretion or inhibition of cytokines, depending on the type of stimulus given.

At last the effect of MCTs on allergic immune responses is investigated. Not much information is known about this subject, and existing literature is contradictory. In most studies experimental setups are different and diverse medium chain fatty acids are used.

To summarize, MCFAs are found to influence immune responses on different levels. However, the different results are not consistent and in most cases contradictory. It can be concluded that little is known about the effect of medium chain triglycerides on immune cells and function and more research is needed.

Introduction

Triglycerides are composed of a glycerol backbone with three fatty acid chains (*Fig. 1)*. They can be divided into two groups: triglycerides which contain saturated fatty acids, and triglycerides containing unsaturated fatty acids. In unsaturated fatty acids, there are one or more double bonds between the carbon atoms. This in contrast to saturated fatty acids in which all places at the carbon atoms are saturated with hydrogen. Since in triglycerides the three fatty acids, bound to the glycerol backbone are usually different, many kinds of triglycerides are known. Fatty acids found in plants and animals typically contain even numbers of carbon atoms.

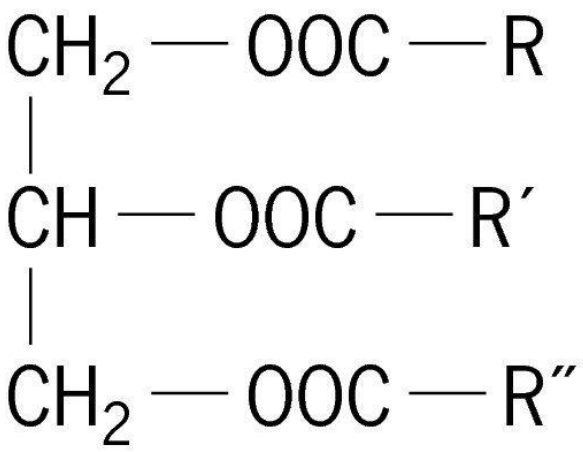

Figure 1; Composition of a triglyceride. A glycerol backbone (left) bound to three fatty acids (right) [1].

Besides discrimination in saturation degree, a distinction can be made also based on length of fatty acids, namely short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and long chain fatty acids (LCFA). SCFAs have a length of two to five carbon atoms. They are traditionally present in bovine milk and butter fat.. On average, MCFAs are fatty acids with a length of six to twelve carbon atoms, such as hexanoic acid (C6), octanoic acid (C8) or decanoic acid (C10). However, different definitions are in use. In some studies fatty acid with a length of five till nine carbon atoms are considered as MCFAs, while in other studies fatty acids with a length of eight till twelve carbon atoms are considered as MCFAs. Triglycerides containing only MCFAs are called medium chain triglycerides (MCTs). Also triglycerides consisting of a combination of SCFAs, MCFAs and LCFAs exist.

MCTs were introduced in the 1950s as a special energy source in clinical nutrition for patients suffering from fat malabsorption, severe hyperchylomicronemia and pancreatic insufficiency [2, 3].

Uptake of MCT and LCT

Due to the short chain length, MCTs can be absorbed from the intestinal lumen while being intact, whilst other triglycerides, such as LCTs must be broken down into smaller fatty acids before they can be absorbed and used as energy source. For this reason it is thought that MCTs might have unique properties enhancing human health [4].

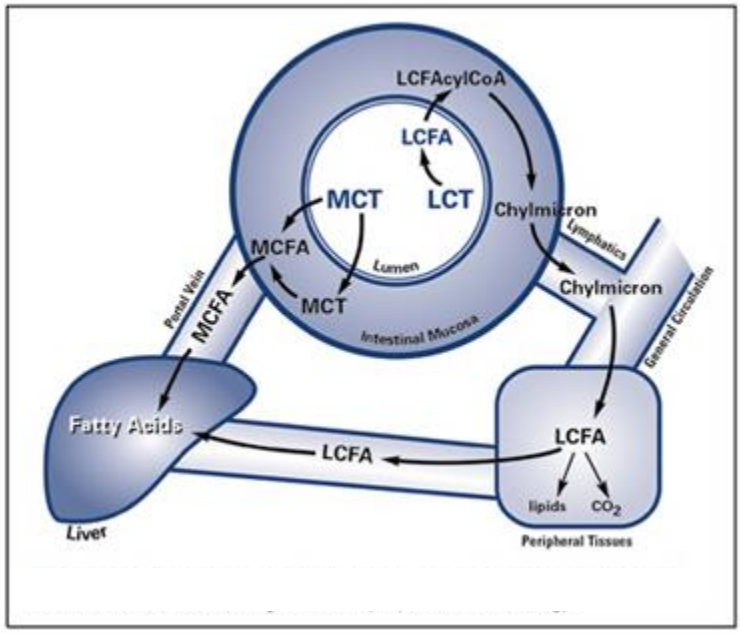

Figure 2; Absorption of MCT and LCT in the intestine [5]. MCTs are degraded into medium chain fatty acids (MCFAs) in the intestinal mucosa and transported to the liver via the portal vein. LCTs are degraded in long chain fatty acids (LCFAs) and transported in chylomicrons via the lymphatic system.

MCTs are hydrolyzed in the small intestine and taken up as MCFAs [6]. MCFAs bind to albumin and are transported to the liver via the portal vein (*Fig. 2)*. This in contrast to LCTs, which first have to be degraded into long chain fatty acids before being absorbed. Since LCFAs are not soluble they are incorporated into chylomicrons and transported in the lymph fluid present in the lymphatic system. Via the lymphatic system they reach the liver. In the liver fatty acids from MCFAs and LCFAs are broken down and LCFAs might be stored as fat, whilst MCFAs might be immediately used as energy.

In the liver MCFAs rapidly undergo β-oxidation in the mitochondria. A process by which energy is released (*Fig. 3)* [7]. Furthermore, binding of MCFAs to Acetyl-CoA, which enters the Krebs cycle, results in excretion of citrate into the cytoplasm, resulting in synthesis of Acetyl-CoA (outside the mitochondria), which is necessary for binding of long chain fatty acids (LCFAs). The CoA part is replaced with carnitine which allows the LCFAs to enter the mitochondria. So, in contrast to MCFAs, LCFAs do not easily enter the mitochondria. Due to the fast absorption and hydrolisation of MCTs they are quickly metabolized. As a result, energy derived from MCTs is used direct by organs and muscles, instead of being stored as fat [8].

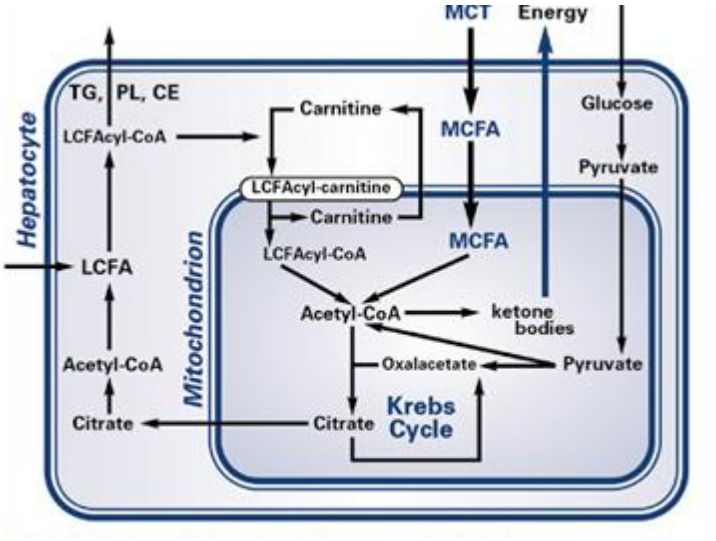

Figure 3; Conversion of MCT and LCT into energy in hepatocytes [5].

Different types of MCTs are used in different preclinical or clinical studies. In most studies coconut oil is used as MCT source. It contains high amounts of MCTs [9]. However, there is also a difference between original, virgin MCTs, and the processed partially hydrogenated and fully hydrogenated MCTs. Hydrogenation is often used to saturate unsaturated fatty acids. By hydrogenating fatty acids, the melting point increases[10]. Virgin MCTs contain low amounts of saturated fatty acids, while fully hydrogenated MCTs contain high amounts of saturated fatty acids. When fatty acids are partially hydrogenated trans-fats can be generated. Trans-fats can increase the levels of LDL cholesterol and lowers levels of HDL cholesterol. Since LDL is associated with various health problems such as coronary artery disease, trans-fats are considered as 'bad' fats. In most of the in this thesis described studies it is not mentioned which type of MCTs is used, or why this type is

used. For this reason no conclusion can be drawn about the different types of MCTs used and their specific effects.

MCT and energy metabolism

Several studies suggest that an increase in MCT intake enhances satiety and therefore a decrease in energy consumption. For this reason it is believed that MCTs are more effective in inhibiting food intake compared to LCTs [11-16]. Moreover, since MCTs are not stored as fat, but the energy is released immediately, consumption of MCTs could be related to lower rates of fat deposition. This all might contribute to a promotion of weight loss [17, 18]. *Fushiki et al*. also described the positive effects of MCTs on energy production and expenditure caused by an increased production of key metabolic enzymes involved in the Krebs cycle [19].

Other properties of MCTs

Besides inhibiting food intake and lowering fat deposition, MCTs are thought to have many other properties. *Shea et al.* show that the intake of MCTs may help alleviate pain from chronic pancreatitis [20]. MCTs are also found to be beneficial against ageing of the brain. After binding of MCFAs to Acetyl-CoA when undergoing β-oxidation ketone bodies are formed. Besides glucose, ketone bodies are the only fuel for the brain. It is postulated that ketone bodies might counter the ageing of the brain [21]. Moreover, MCFAs have anti-coagulation properties [22], and they have been shown to lower cholesterol in serum and liver [21], resulting in a beneficial effect on atherosclerosis prevention.

MCTs in diet

As described, MCFA are indicated to have a lot of positive health benefits. Therefore presence of MCTs in food might be beneficial. However, MCTs are not very common found in our food. Some natural sources of MCTs are coconut and park kernel oil, of which over 50% of total fatty acids is MCFAs [23]. High concentrations of MCFAs are found in milk from mice, rats, rabbits, goats, horses and elephants. Cow's milk, sheep milk and human breast milk also contain MCFAs but in a lesser extent (*Fig. 4*) [24]. The overall percentage of MCTs in human milk is approximately 15% of total fatty acids [25]. Infant formulas must be designed to be similar to human breast milk [26]. Therefore, also MCFAs must be present in infant formulas. Cow's milk and vegetable oil are the main fat sources in infant formulas. The structures of triacylglycerol molecules in these sources

differ from the structures of triacylglycerol molecules in human milk [27]. For this reason often extra MCTs are added to infant formulas. MCT levels in infant milk formula might vary between 5% and 33% of the total fatty acid content.

Fatty acid	MCTAG oil	Coconut oil	Palm-kernel oil	Human milk	Horse milk	Rat milk	Cows' milk	Goats' milk	Sheeps' milk	Rabbit milk	Sows' milk
4 : 0							4·8	3·1	4·0	0·0	0·1
6 : 0	1·0–2·0	0·5	0·3	0·1			2·2	2·5	1·7	0·0	0·1
8 : 0	65·0–75·0	8·0	3·9	0·2	8·0	8·7	1·3	2·9	1·4	34·3	0·0
10 : 0	25·0–35·0	6·4	4·0	1·0	17·1	23·6	2·9	10·2	2·6	21·4	0·0
12 : 0	2·0	48·5	49·6	4·9	14·3	15·4	3·3	6·1	1·6	1·2	0·0
14 : 0		17·6	16·0	5·6	8·7	10·6	10·8	12·5	5·0	1·0	4·0
16 : 0		8·4	8·0	20·3	15·3	16·2	26·2	28·6	23·3	11·7	36·6
16 : 1				3·4	4·0	1·5		2·6	1·3	1·2	10·5
18 : 0		2·5	2·4	7·5	1·2	2·4	10·8	6·2	19·2	2·1	5·9
18 : 1		6·5	13·7	33·6	8·3	14·5	24·1	21·6	33·5	12·6	32·3
18 : 2		1·5	2·0	12·6	6·1	7·1	2·4	3·6	3·5	13·8	8·4
18 : 3				1·0	4·3		1·1	0·0	2·9	0·7	1·0

MCTAG, medium-chain fatty acid-containing triacylglycerols.

Figure 4; Composition of milk from different species (g/100g fatty acids) [24].

Thesis Question

MCT and the immune system

Recent work has demonstrated that fatty acids play a key role in immune responses to dietary antigens. The effect of fatty acids on immune responses can be obtained in different manners, for example via modulation of the intestinal permeability or influence on intestinal epithelial cytokine expression. [28, 29]. How fatty acids cause these effects and which fatty acids are responsible is not exactly known. The purpose of this thesis is to sort and list existing literature about the effect of MCTs or MCFAs on immune cells or the immune system.

Modulation of the immune system using MCTs

Epithelial lining

In 1991 *Kvietys et al.* showed the effect of LCFAs on the epithelial cell lining [30]. The jejunum from rats which either obtained perfusion with sodium taurocholate or with sodium taurocholate plus oleic acid, which is a LCFA was studied. LCFAs caused dramatic damage to the microvilli, resulting in disruption of the epithelial monolayer [30]. Since dietary antigens can easier be absorbed, this increase in intestinal permeability may result in an allergic sensitization [31]. In this way LCFAs may have an indirect influence on immune function. Besides LCFAs, also MCFAs have been shown

to influence intestinal permeability. They do so via a disruption of intestinal tight junction barrier functions. These alterations are particularly caused by capric (C10) and lauric (C12) acid [32].

In 1984 *Naccache et al.* reported specific effects of MCFAs on neutrophil function [33]. Rabbit peritoneal neutrophils were used, and MCFAs were found to stimulate neutrophil aggregation. Aggregated neutrophils can stimulate an immune response, causing tissue damage [33]. In 2002 *Wanten et al*. also studied the effect of MCFAs on neutrophil function [34]. Neutrophils, isolated from human blood were checked for activation after incubation with either unsaturated MCFAs or saturated fatty acids. Neutrophil activation was measured by production of oxygen radicals. An increase in neutrophil activation was found after incubation with MCFAs, not after incubation with saturated fatty acids [34]. MCFAs were also found to increase IL-8 secretion by Caco-2 cells [35]. An increase in the chemokine IL-8 might result in enhanced mucosal infiltration by neutrophils [35]. Because IL-8 is a cytokine which also activates neutrophils, increased IL-8 secretion levels results in not only chemotaxis, but also in an increase in activated neutrophils. This is thought to contribute to the mucosal infiltration by neutrophils resulting in intestinal inflammation. However, study results are inconsistent. *Ohta et al.[36]* and *Hoshimoto et al. [29]* both found a decrease in IL-8 production respectively in rat intestinal cells and Caco-2 cells after incubation with MCFAs. Explanation could be a difference in experimental set-up (*in vivo* versus *in vitro*) or a difference in MCFAs used.

Inflammation

Wang et al. studied the role of GPR84 on immune function *[37]*. GPR84 is a G protein-coupled receptor, which acts as a receptor for MCFAs with a length of 9-14 carbon atoms. GPRs are composed of seven trans membrane domains and are one of the biggest gene families identified so far. They can be activated by a large range of ligands. Another GPR which is associated with fatty acids is GPR40. GPR40 acts as a receptor for MCFAs and LCFAs and it mediates fatty acid induced insulin secretion from pancreatic β cells [38, 39]. GPR40 is mostly found in the pancreatic β cell whilst GPR84 is mostly expressed in activated monocytes/macrophages and in neutrophils. Since GPR84 is mostly expressed in immune cells, it is likely that MCFAs might modulate the function of these cells. Binding of MCFAs to GPR84 results in an increased calcium mobilization, an inhibition in 3' 5'-cyclic AMP production and a stimulation of [^{35}S] guanosine 5'-O-(3-thiotriphosphate) binding [37]. However, how these influences immune function is only limited known. MCFAs amplify lipopolysaccharide-stimulated production of IL-12 by binding to GPR84 [37]. IL-12 is a pro-

inflammatory cytokine which plays an important role in stimulating cell-mediated immunity. It also maintains T-helper 1 (T_H1) responses and inhibits T-helper 2 (T_H2) responses. These T_H1 reactions might have harmful consequence in several auto immune and inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis [40, 41]. From this all it is concluded that MCFAs might, under certain circumstances, influence T_H1/T_H2 balance which could have consequences relating to several auto immune diseases or other immune related diseases. However, since T_H1 responses also result in a normal, healthy, defense against pathogens, an increase in T_H1 response does not necessary has to be harmful [42].

Kono et al. showed a positive effect of MCTs on immune response to antigens [43]. They found that presence of MCTs resulted in an increase in IgA secretion in the ileum after stimulation with LPS. Rats were given MCTs or corn oil daily for one week and LPS or saline was administered via the tail vein. IgA plays an important role in the mucosal immune system [43]. It binds to specific antigens present on the surface of bacteria, viruses and other compounds, preventing attachment to the mucosal surface. Moreover, IgA also prevents against intestinal injury after LPS administration [43]. *Kono et al.* also measured different mRNA expression levels of pro- and anti-inflammatory cytokines. mRNA expression levels of IgA-stimulating cytokines IL-6 and IL-10 was increased in the ileum of rats receiving the MCT diet. Increased expression of pro-inflammatory cytokines and chemokines in the ileum, such as TNFα, IL-18, MCP-2 and MIP-1 was blunted in the presence of MCFAs [43]. *Yoshida et al.* measured IL-6 secretion after addition of MCFAs and LCFAs to rat IEC-6 cells in combination with IL-1β and TGFβ as stimulus [28]. They did not find an increase in IL-6 secretion after incubation with MCFA. However, they did find increased levels of IL-6 after incubation with LCFAs [28].

There are indications that fatty acid might modulate dendritic cell (DC) responses. DCs are antigen presenting cells, which play an important role in initiating immune reactions by stimulating T-lymphocytes. DCs have an important role in the intestine, initiating mucosal immune responses. *Tsuzuki et al.* found that both LCFAs and MCFAs maintained phagocytic function of DCs after stimulation with TNFα. However, only exposure of DCs to LCFAs, but not MCFAs, led to down regulation of antigen presentation *[44]*. LCFAs significantly suppressed expression of MHC class-II molecules by DCs. Also expression of co-stimulatory factors was suppressed by LCFAs. This results in a decreased antigen presentation ability of DCs, which might induce dysregulation of lymphocyte migration abilities which will induce abnormal lymphocyte trafficking [44].

Nanji et al. tested the effect of MCFAs or fish oil on inflammatory and fibrotic changes after continued ethanol administration. After administration of MCFAs histological improvement was observed. Also decreased levels of endotoxin and lipid peroxidation were measured in liver samples, resulting in absence of NFκB activation and reduced pro-inflammatory cytokine TNFα and COX2 levels. COX2 results in induction of prostaglandin synthase, by which inflammation increases *[45]*.

Decanoic acid (a MCFA) was found to bind and activate peroxisome proliferator-activated receptor γ (PPARγ). PPARs are nuclear transcription factors. PPARγ is mainly expressed in adipose tissue, monocytes/macrophages, dendritic cells and B and T cells. It is thought to trigger adipocyte differentiation and plays a key role in glucose and lipid metabolism. *Malapaka et al.* describe that the MCFA decanoic acid can bind and activate PPARγ and in this way reduces blood glucose levels *[46]*. Activation of PPARγ was measured using a plasmid in which the luciferase was under control of the PPARγ gene. Since diabetic patients often have high blood glucose levels decanoic acid might be a potential drug for diabetic patients. PPARγ is not only associated with diabetes type II but it also links with immune modulation. Previous studies have shown that activation of PPARγ leads to a decrease in pro-inflammatory cytokines like IFNγ and TNFα, and an increase in anti-inflammatory cytokines such as IL-4 and IL-10 [47, 48]. It is understood that activation of PPARγ leads to an increased T_H2 cytokine secretion [47]. *Malapaka et al.* found that decanoic acid activates PPARγ but modulation on immune parameters was not tested *[46]*.

Allergies

In 2012, *Li et al.* described that MCTs in diet promote oral allergic sensitization and anaphylaxis to peanuts in mice [3]. This suggests that MCTs have an immune modulatory function, which is, according to the findings of *Li et al.* a negative effect. Mice were fed either a MCT or a LCT based peanut diet or control diet for four weeks. After this, blood was collected for detection of levels of IgE and IgG. Also T_H2-cytokine expression levels (TSLP, IL-25, IL-33) were measured. These different cytokines have previously been implicated in allergic diseases [49]. A significant induction of intestinal epithelial Th_2-cytokine expression levels in the jejunum was detected. Besides this, an increase in antigen absorption was observed in the Peyers patches. The Peyers patches are lymph nodules in the wall of the small intestine, and produce antibodies when interaction with antigens occurs. These effects were not visible in mice receiving a LCT based diet. It was concluded that

MCTs might promote oral allergic sensitization. More research is needed however to confirm this effect. *Wang et al. [50]* describe that the presence of LCTs in the diet results in an increase of antigen absorption. The presence of MCTs in diet does not show this effect. OVA absorption is measured in mice fed LCT or MCT enriched diets. It was found that absorption of OVA into the lymph nodes and blood was significantly enhanced when LCT, but not MCT was present. Since presence of LCT leads to secretion of chylomicrons, OVA is easier absorbed and transported throughout the body. Chylomicrons are phagocytized by macrophages, facilitating antigen capture [51].

As previously described, MCTs might bind and activate PPARγ. PPARγ are indicated in contrast with the study of *Li et al*. to be associated with allergic reactions. *Dahten et al. [52]* showed that activation of PPARγ in atopic dermatitis patients resulted in a significantly reduced onset of eczematous skin lesions. Skin thickness was decreased and there was a significant reduction of lymphocytes and mast cells.

Combined results

All articles used to examine the effect of MCTs on immune responses are summarized in *table 1* and *table 2.* Study set up, results and conclusion are present in these tables.

Table 1; *In vitro* studies used in this thesis to investigate the effect of MCTs on immune responses.

Study	Methods	Effects	Conclusion
Hoshimoto [29] ***Caco-2 cell line***	Caco-2 cells +/- fatty acids.	Caprylic acid reduces IL-8 production.	The pro-inflammatory capacity of LCFAs is larger than MCFAs.
Lindmark [32] ***Caco-2 cell line***	Caco-2 cells +/- MCFAs.	Capric and Lauric acid influence tight junction barrier function.	Absorption is enhanced by MCFAs, causing increased tight junction permeability.
Malapaka [46] ***Cos-7 cell line***	Cos-7 cells containing a plasmid with PPARγ gene + decanoic acid.	Decanoic acid binds and activates PPARγ.	Decanoic acid is considered to be a modulator of PPARγ.
Tanaka [35] ***Caco-2 cell line***	Caco-2 cells + different fatty acids.	MCFAs stimulate IL-8 production	MCFAs stimulate IL-8 production causing an increase in neutrophil infiltration.
Yoshida [28] ***IEC-6 cell line***	IEC-6 cells were co-cultured with LCFAs or MCFAs.	Presence of LCFAs, but not MCFAs increased IL-6 levels.	LCFAs stimulate cytokine release under conditions of inflammatory stimulation.

Table 2; *In vivo* studies used in this thesis to investigate the effect of MCTs on immune responses.

Study	Methods	Effects	Conclusion
Dahten[52] ***Mice***	PPARγ agonist was administered in mice with AD-like skin lesions.	Reduced infiltration of lymphocytes and mast cells.	Activation of PPARγ might inhibit allergic immune responses.
Kono [43] ***Rats***	Rats were fed corn oil or MCT and LPS or saline was administered via tail vein.	LPS+MCTs increase IL-6 and IgA secretion.	MCTs influence immune responses to LPS and this was protect the gut.
Kvietys [30] ***Rat***	Structural changes in epithelial lining after perfusion of jejunum with LCT.	Changes of the epithelial lining are visible after perfusion with LCT.	Oleic acid causes disruption of the epithelial barrier.
Li [3] ***Mice***	Gavage of peanut protein + MCT/ LCT.	MCTs increase IL-25, IL-33 and T_H2 responses.	Stimulation of T_H2 response by MCTs results in allergic sensitization.
Naccache [33] ***Rabbit***	Neutrophil suspension + different fatty acids.	MCTs might stimulate aggregation and degranulation of neutrophils.	MCFAs are capable of modulating immune function
Nanji [45] ***Rats***	Rats were fed MCTs or fish oil combined with ethanol.	MCTs caused decreased levels of endotoxin and lipid peroxidation.	MCTs reduce alcohol-induced necrosis and inflammation.

Ohta [36] ***Rat***	Rats with ileitis in the intestine were fed MCFAs or LCFAs.	MCFAs reduce IL-8 production levels.	MCFAs reduce intestinal damage in ileitis rats due to a reduction in IL-8 secretion.
Saubermann [47] ***Mice***	PPARγ agonists are administered to PPARγ +/- mice.	Activation of PPARγ results in increased anti-inflammatory cytokines.	PPARγ shifts immune response from a T_H1 response to a T_H2 response.
Tsuzuki [44] ***Rats***	Dendritic cells + MCFA/LCFA.	LCFAs caused decreased expression of MHC-II and decreased antigen presentation ability by DCs.	Exposure to LCFAs might modulate immune function of dendritic cells.
Wang [37] ***Human + Mice***	GPR84 was cloned by PCR from human and mice bone marrow cDNA.	MCFAs bind GPR84 causing increased production of IL-12.	MCFAs may affect T_H1/T_H2 balance in a GPR84-dependent manner.
Wang [50] ***Mice***	Absorption of antigens after gavage of OVA + LCT or MCT.	LCT resulted in increased absorption of OVA into blood.	Formation of chylomicrons affects absorption and dissemination of dietary antigens.
Wanten [34] ***Human***	Neutrophil suspension + MCT/LCT.	MCTs caused a significant oxygen radical production.	MCTs might stimulate neutrophil activation.

Discussion

MCFAs are indicated to have many beneficial effects. Since MCFAs and LCFAs are differentially absorbed, transported and used by the human body, these fatty acids are likely to have different effects. MCFAs are thought to reduce food consumption by enhancing satiety. Consumption of MCFAs might lead to weight loss. Moreover, also influences of MCFAs on the ageing of the brain and lowering of cholesterol was found. The focus of this thesis is to describe the current knowledge on the effect of MCTs on the immune system. However, limited literature was found. Moreover, the literature about the specific role of MCTs is contradictory.

Literature indicates that MCFAs might influence many different aspects of the immune system, starting with their role on the epithelial lining of the intestinal lumen. Both LCFAs and MCFAs are indicated to influence barrier functions, but in different ways. LCFAs might disrupt the epithelial lining (ex vivo data), whilst MCFAs affect in a more specific way intestinal tight junction function (in vitro data) [30, 32]. Whether LCFAs, besides the epithelial cells, also influence the tight junction barrier function is not examined. By alterations of the epithelial lining the first defense mechanism against immune response is attacked. MCFAs might, by alteration of the tight junction function, cause a greater chance of antigens and pathogens entering the intestinal mucosa.

Besides an effect on epithelial lining, MCFAs might also affect cytokine secretion by epithelial cells. *Tanaka et al*. and *Hoshimoto et al.* both measured IL-8 levels in Caco-2 cell lines [29, 35]. *Tanaka et al*. found an increase in IL-8 excretion levels, whilst *Hoshimoto et al.* saw a lowering in IL-8 levels. This can be explained by the fact that different MCFAs are used. Whilst *Tanaka et al.* looked at the effect of capric acid, *Hoshimoto et al.* studied caprylic acid. Also the experimental setup was different. In both cases Caco-2 cells were triggered using IL-1β, but *Tanaka et al.* added the fatty acids in the apical part of a transwell system, cultured with Caco-2 cells, whilst *Hoshimoto et al.* cultured the cells in 24-wells plate and added the fatty acids in the supernatant. This all can contribute to the different results found in both studies. Since the transwell-system is more physiologically correct it is more likely that this data is correct and presence of MCFAs leads to an increase in IL-8. IL-8 is a cytokine which attracts neutrophils. More IL-8 present will lead to an increase in neutrophils. The effect of MCFAs on neutrophil function was showed in two articles which found enhanced neutrophil activation and aggregation [33, 34]. Both measured this in an *in vivo* set up of a neutrophil suspension. From this we can conclude that MCFAs are likely to increase

IL-8 secretion, and an association is made between presence of MCFAs and neutrophil activation, resulting in more inflammation.

Kono et al. describe the administration of MCTs in combination with LPS, a stimulus for T_H1-responses, resulting in a lowered T_H1 response [43]. Different pro- and anti-inflammatory cytokines are measured in serum and gut samples, amongst which is IL-6. IL-6 is an IgA–stimulatory cytokine. IgA binds in the intestine to specific antigens present on the surface of bacteria, viruses and other compounds, preventing attachment to the mucosal surface. *Yoshida et al.* measured IL-6 secretion after administration of MCTs, but did not find an influence of MCFAs on IL-6 secretion. Different explanations are present for this inconsistency in results. *Kono et al.* tested the effect of MCTs in an *in vivo* setup, while *Yoshida et al.* did this in an *in vitro* set up, using an IEC-6 cell line. Also the stimulus in both cases is different. *Kono et al.* used LPS as stimulation, *Yoshida et al* used IL-1β and TGFβ. This all can explain the difference in result which was found. We can conclude that MCFAs might, in the presence of LPS, a bacterial fragment, increase IL-6 secretion, leading to an increase in IgA.

Kono et al. did not only find an increase in IL-6 levels, they also show a decrease in pro-inflammatory cytokines which usually are correlated with a T_H1 response [43]. This decrease in pro-inflammatory cytokine levels results in diminished T_H1 response. Since *Kono et al.* used LPS as stimulus, which is a stimulus for T_H1 responses, an increase in T_H1-correlated cytokines would be expected. Addition of MCFAs impair T_H1 responses [43]. *Nanji et al.* looked at the immune modulatory capacity of MCFAs in a rat model of ethanol induced intestinal inflammation [45]. They found that presence of MCFAs leads to a reduced NFκB activation and reduced secretion of TNFα and COX2 after stimulation with ethanol. This was measured in serum and liver samples. A decrease in pro-inflammatory cytokines results in a lowered T_H1 response. *Malapaka et al.* also showed that presence MCFAs leads to a decrease in pro-inflammatory cytokines such as TNFα and IL-10. This is caused by activation of PPARγ. Contradictory is the result reported by *Wang et al.,* about an increase in IL-12 secretion after stimulation with LPS, leading to an increase in T_H1 response [37]. This difference in results can be explained by the fact that different experimental set-ups are used and different MCFAs are tested. *Wang et al.* measured capric acid, undecanoic acid and lauric acid, which are not tested by *Kono et al., Nanji et al.* and *Malapaka et al.*. Although the different experiments did not measured the same cytokines, we can conclude that addition of MCFAs combined with a stimulus might lead to a decrease in inflammatory responses. Since *Nanji*

et al. found a decrease in NFκB activation it might be suggested that this reduction causes the decrease in pro-inflammatory cytokines.

No clear effect of MCFAs on DC response is found in a model using DCs isolated from lymph from rats[44]. The phagocytic function is maintained after in vitro administration of MCFAs. However, this is also maintained after administration of LCFAs, concluding that the tested fatty acids do not modulate phagocytosis. Exposure of DCs to LCFAs reduced antigen presentation. This is caused by down regulation of MHC-II by LCFAs and leads to abnormal lymphocyte trafficking, causing reduced tolerance to food antigens. From this we can conclude that more MCFAs is positively correlated to antigen presentation by DCs.

Results coming from the study from *Wang et al.* showed that presence of LCFAs resulted in chylomicron formation, which resulted in increased antigen uptake. Since chylomicrons are captured by macrophages, it is proposed the normal immune responses against antigens might occur and tolerance is induced. However, since MCFAs do not stimulate chylomicron formation, this effect was not visible when studying MCFAs.

The main goal of this thesis was to identify the effect of MCTs on allergy induction. Not much information is known about this subject. *Li et al.* described that MCTs promote allergic sensitization against peanut proteins [3]. They discovered a suppressed antigen absorption into the blood when MCTs are present. This way no normal immunologic response against these antigens can occur. They also found an increased T_H2 cytokine response, which causes allergic responses [53]. *Wang et al.* measured T_H2 cytokine levels after LPS stimulation and found a decrease in T_H2 cytokine levels, suggesting that this would lead to a decrease in allergic response [37]. However, this is contradictory to the results found by *Li et al.*. Since *Li et al.* used a MCT-oil it is not known which MCFAs caused the effect they found. K*ono et al.* found an increase in IgA, which is related to a decrease in immune responses, since IgA prevents antigens from crossing the epithelium, and causing a better first line of defense. This all results in a lowered change of developing allergic reactions [53-55]. *Malapaka et al.* found that presence of MCFAs activate PPARγ. According to *Dahten et al.* which measured allergic immune responses in the skin of a murine model, activation of PPARγ might lead to a reduced allergic reaction [52]. However, *Saubermann et al*. found that activation of PPARγ leads to an increase in IL-4 and IL-10 [47]. The difference in study design might explain this difference in results found. *Saubermann et al.* investigated PPARγ +/- mice and its development of colitis, while *Dahten et al.* looked at allergic immune responses in the skin in Balb/C

mice. From this all it is concluded that results found about the effect of MCFAs on allergic responses are quite contradictory. In all cases a stimulus is used to obtain an immunologic response, but experimental setups are different. In most cases, also different MCFAs are used. So, with the known data no conclusion can be drawn about the effect of MCTs on allergic responses.

To summarize, MCFAs are found to influence immune responses on different levels. However, the different results are not consistent and in most cases contradictory. It can be concluded that little is known about the effect of medium chain triglycerides on immune cells and function and more research is needed.

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