# Effect of a 12-Week Mediterranean Diet on Rectal Submucosa White Adipose Tissue (WAT) Inflammation and Fat Droplet Size in Patients with an Advanced Adenoma or Early colorectal Adenocarcinoma: A Pilot Study
# The minivan- study

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Primary Objective: - To examine whether a 12-week Mediterranean Diet (MeD) intervention reduces white adipose tissue (WAT) inflammation or fat droplet size in the rectal submucosa in patients with BMI >= 25 kg/m2 who have undergone an endoscopic...

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| **Ethical review** | Approved WMO |
| **Status** | Recruiting |
| **Health condition type** | Gastrointestinal neoplasms malignant and unspecified |
| **Study type** | Interventional |

## Summary

### ID

NL-OMON53454

**Source**
ToetsingOnline

**Brief title**
Effect of a Mediterranean Diet on Rectal Submucosa WAT Characteristics

### Condition

- Gastrointestinal neoplasms malignant and unspecified

**Synonym**
bowel cancer; colorectal carcinoma, colorectal andenoma, rectal adenoma, rectal carcinoma

**Research involving**
Human

## Sponsors and support

**Primary sponsor:** Leids Universitair Medisch Centrum
**Source(s) of monetary or material Support:** Leids Universitair Fonds

## Intervention

**Keyword:** colorectal adenoma, colorectal carcinoma, Mediterranean diet, nutrition

## Outcome measures

### Primary outcome

- Mean reduction in fat droplet size between the pre- and post-MedD biopsies of the rectal submucosa in participating patients.

- Mean reduction in number of crown-like structures the pre- and post-MedD biopsies of the rectal submucosa in participating patients.

- Mean change in the M1:M2 macrophage ratio between the pre- and post-MedD biopsies of the rectal submucosa in participating patients.

### Secondary outcome

- Relationship between baseline rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and body composition measures in participating patients.

- Relationship between the pre- and post-MedD changes in rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and body composition measures in participating patients.

- Relationship between baseline rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and homeostatic model assessment insulin resistance (HOMA-IR) measures in participating patients.

- Relationship between the pre- and post-MedD changes in rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and HOMA-IR measures in participating patients.

- Relationship between baseline rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and liver fat (as a measure of ectopic fat stores) in participating patients.

- Relationship between the pre- and post-MedD changes in rectal submucosa WAT characteristics (number of CLS, M1:M2 macrophage ratio, and fat droplet size) and liver fat (as a measure of ectopic fat stores) in participating patients.

# Study description

**Background summary**

Globally, colorectal cancer (CRC) is the third most commonly diagnosedmost diagnosed cancer and the second most common cause of cancer death.1 The number of new cases is projected to increase from more than 1.9 million of new cases in 2020 to more than 3.5 million by 2040. The Netherlands has the fourth highest age-standardized incidence rate in Europe, at 41.0 cases per 100 000 persons behind Hungary (45.3 cases), Slovakia (43.9 cases), and Norway (41.9 cases).2 The development of CRC is multifactorial and involves interactions between genetics, epigenetics, and environmental and lifestyle-related factors such as poor diet.2,17

Overweight and obesity are important risk factors for CRC.3,4 However, the mechanisms underlying the association between overweight/obesity and the development of CRC are still not fully understood. However, current research evidence suggests that chronic-low grade inflammation in white adipose tissue (WAT) and in peripheral target organs plays a key role. In particular, excessiveExcessive accumulation of visceral fat due to overnutrition is associated with changes that lead to an inflammatory state: pathogenic hypertrophy and dysfunction of adipocytes; increased levels of free fatty acids; and deregulation of adipokines (eg, adiponectin and leptin), pro-inflammatory mediators, growth factors, and reactive oxygen species.5

Consequences of an inflammatory state in the adipose tissue microenvironment

WAT consists of many different cells such as adipocytes, immune cells, fibroblasts, and endothelial cells, which all play a role in maintaining tissue homeostasis. Together, these cells comprise the adipose tissue microenvironment (ATME).

Under normal weight conditions, the ATME exists as an anti-inflammatory immune state. However, when (excessive) weight gain occurs and accumulates particularly around the viscera such as the large intestine,18 adipocyte hypertrophy and hyperplasia occur. Adipocyte hypertrophy can lead to adipocyte death, which triggers an (innate) immune response and shifts the ATME towards a pro-inflammatory state. The pro-inflammatory state is characterized by an increased release of pro-inflammatory cytokines [eg, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1-beta] and adipokines (eg, adiponectin and leptin). CD4+ T cells and macrophages also accumulate.19,20 Through cellular crosstalk, this obesity-driven inflammatory state may increase tumor growth and progression as visceral adipose tissue can exist as peritumoral fat.21,22

Adipocytes and inflammatory cells are sources of potential carcinogens. In breast cancer, for example, the pro-inflammatory adipokine, leptin, was identified as an important biomarker of breast cancer prognosis. Leptin and its receptor are overexpressed in breast cancer cells compared to normal mammillary epithelial cells23 and by binding to its receptor, leptin activates STAT3, ERK, and P13K/AKT pathways, which triggers breast cancer cell proliferation.24,25 Compared with adjacent normal colon tissue, both leptin and its receptor have also been found to be overexpressed in CRC tissue.26

Crown-like structures

When hypertrophic adipocytes in the WAT begin to die, their cellular content is released into the ATME. This cellular content acts as a signal for macrophages to surround and scavenge the dying adipocytes, thereby creating so-called crown-like structures (CLS).6-10 Multiple receptors on the membranes of macrophages (eg, toll-like receptors) are stimulated and lead to the activation of inflammatory pathways. The presence of CLS in obese WAT is viewed as a biomarker of WAT inflammation.10 In cancer patients, CLS have been linked to worse prognosis.

The association between WAT inflammation and cancer has previously been shown for breast,27-29 prostate,30 and tongue31 cancers. For example, prevalence of CLS in breast adipose tissue was 40-50% in breast cancer patients,27,28 with a higher prevalence in overweight and obese patients compared to lean patients. Moreover, there existed a linear correlation between adipocyte size and CLS density in breast adipose tissue.27 Mechanistically, this may be explained by hypertrophy leading to adipocyte death, which triggers accumulation of macrophages into CLS. Thus, next to systemic elevations in pro-inflammatory factors, obesity may also promote tumorigenesis through local adipose tissue inflammation. In case of prostate cancer, prevalence of periprostatic WAT inflammation was around 50%, and periprostatic WAT inflammation was associated

with a higher BMI, a larger adipocyte size and higher grades of tumors.30

M1 and M2 macrophages

Macrophages may be divided into pro-inflammatory M1 or anti-inflammatory M2 classifications. In the context of WAT inflammation, both M1 and M2 macrophages appear to have a role in the pathogenesis of CRC. First, findings from a combined retrospective cohort study with a mouse model of CRC, indicated that CRC patients who consumed >100g red meat daily over the past year (which was considered an HFD) had an increased expression of MCP-1 and its receptor CCR2 and an increased incidence of more advanced stages of CRC. The HFD in mice induced microbial dysbiosis, promoted intestinal carcinogenesis, upregulated the MCP-1/CCR2 axis, and recruited M2 tumor-associated macrophages.32

Second, Pinto et al33 found the predominance of anti- or pro-inflammatory macrophages can differ depending on tumor-associated location and tumor stage. For instance, anti-inflammatory (CD163+) macrophages were predominant at the tumor invasive front and pro-inflammatory ones (CD80+) in the adjacent normal mucosa. Also, anti-inflammatory macrophages were more abundant in T2 tumors and pro-inflammatory ones in T1 tumors. In T3 tumors, a higher overall macrophage infiltration and a lower pro-inflammatory/anti-inflammatory ratio was associated with worse survival. It is hypothesized that when a tumor initially develops, newly recruited macrophages are pro-inflammatory and in later stages, may switch to an anti-inflammatory phenotype.

Third, a study of 20 patients with CRC and mean BMI of 27.2 kg/m2 demonstrated that M2-like phenotype macrophages were predominant in peritumoral visceral adipose tissue and pro-inflammatory M1 macrophages in CLS of visceral and subcutaneous adipose tissue. M2 macrophages may induce angiogenesis in peritumoral tissue, which favors tumor growth. An in vitro co-culture experiment in the same study that used adipocytes and a colon cancer cell line suggested evidence for adipocyte-cancer cell crosstalk in CRC.34

Colorectal submucosal WAT deposits

More recently, next to the visceral and subcutaneous adipose tissue, also colorectal submucosal WAT deposits have been found. This submucosal WAT, inside a tissue normally not accommodating fat, maycan have pathogenic effects. Submucosal fat may act locally as an endocrine and paracrine organ acting on adjacent anatomic organs. Submucosal fat accumulation in the colon and/or rectum may play a role in the metabolic syndrome or even in the development of CRC and is associated with an increased BMI.35 In this study we will take rectum biopsies both from patients in whom the lesions is located in the rectum and in whom it is located outside of the rectum.

The potential of Mediterranean diet for CRC prevention

Since weight gain and the ensuing low-grade inflammation appear to have key roles in obesity-related cancers such as CRC, an anti-inflammatory

Mediterranean diet may represent a relevant strategy to reduce the incidence of CRC.

Indeed, the World Cancer Research Fund (WCRF) reported that diets high in red meat and low in fruit and non-starchy vegetables might increase the risk for CRC. Consumption of vitamin C rich foods, fish, and vitamin D and multivitamin supplements might decrease the risk for CRC.36 A recent meta-analysis concluded that a pro-inflammatory western diet (high intake of meat and processed foods) was associated with an increased CRC risk. Conversely, more *healthy* diets (rich in fruits and vegetables) were associated with a decreased CRC risk.37

Furthermore, a meta-analysis concluded that consuming a more pro-inflammatory diet was associated with a 12-65% increased risk for CRC compared to consuming a more anti-inflammatory diet. This anti-inflammatory diet was characterized by a high intake of plant-based foods and a low intake of animal-based foods.12 A large cohort study also found that more pro-inflammatory dietary patterns were associated with an increased risk for the development of CRC.13

In this pilot study, we aim to investigate whether a 12-week Mediterranean diet (MeD) intervention alters fat droplet size or the degree of inflammation in the rectal submucosa of overweight patients who have undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.

**Study objective**

Primary Objective:
- To examine whether a 12-week Mediterranean Diet (MeD) intervention reduces white adipose tissue (WAT) inflammation or fat droplet size in the rectal submucosa in patients with BMI >= 25 kg/m2 who have undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.

Secondary Objective(s):
- To explore the relationship between rectal submucosal WAT characteristics (inflammation and fat droplet size) and body composition measures (height, weight, waist-hip ratio, bio-impedance) in patients with BMI >= 25 kg/m2 who have undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.
- To explore the relationship between rectal submucosal WAT characteristics (inflammation and fat droplet size) and insulin resistance in patients with BMI >= 25 kg/m2 who have undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.
- To examine the relationship between rectal submucosal WAT characteristics (inflammation and fat droplet size) and liver fat (as a measure of ectopic fat stores) in patients with BMI >= 25 kg/m2 who have undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.
- To assess whether a 12-week MeD intervention alters the ratio between M1 and M2 macrophages in the rectal submucosa of patients with BMI >= 25 kg/m2 who have

undergone an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.
- To explore the relationship between changes in rectal submucosal WAT characteristics (inflammation and fat droplet size) to that of changes in the fecal microbiome of patients with BMI >= 25 kg/m2 who have followed a 12-week MeD intervention after undergoing an endoscopic resection of an advanced adenoma or early (T1) colorectal adenocarcinoma.
- To compare rectal submucosal WAT characteristics (inflammation and fat droplet size) between subjects in whom the colorectal lesion is located within and outside of the rectum.

**Study design**

We will perform a single-center open-label intervention study. The study procedures and the 12-week Mediterranean diet (MeD) intervention are integrated into the standard care timelines for endoscopic resection or endoscopic submucosal dissection (ESD) of an advanced adenoma or early-stage (T1) colorectal adenocarcinoma.

In general, the study comprised 3 phases: baseline & recovery, intervention, and follow-up. Figure 1 provides an overview of the study design and main procedures. First, the baseline & recovery phase consists of the endoscopic resection of the cancerous tissue, baseline biopsy of submucosal tissue, and nonsurgical assessment procedures (ie, baseline blood and fecal samples, anthropometric and body composition measurements, a liver ultrasound elastography, and completion of questionnaires). The standard postoperative recovery period after an endoscopic resection is 3 months. Second, the intervention phase consists of the 12-week MeD intervention. Last, the follow-up phase consists of a postoperative colonoscopy to check healing after the resection and to take a follow-up biopsy of the submucosal tissue. In addition, the nonsurgical assessments will be repeated.

**Intervention**

The intervention is a 12-week, anti-inflammatory Mediterranean diet (MeD). MeD is characterized by the following:14,15
- a high intake of vegetables, fruit, pulses, grains, and nuts
- moderate consumption of yogurt, cheese, egg, poultry, fish, and seafood
- moderate intake of wine with meals
- little or no red or processed meats
- olive oil being the predominant source of fat

**Study burden and risks**

The burden for patients includes the time investment to undergo measurements at baseline, before the start of the intervention, and at follow-up; and to

receive dietary counselling before the start of the intervention and at 4-weeks and 8-weeks.

To minimize the burden for patients, the study procedures are embedded as much as possible within the standard care protocol and timelines for endoscopic resection of of an advanced adenoma or early-stage (T1) colorectal adenocarcinoma. The first 2 biopsies will be performed during the endoscopic resection of the cancerous tissue; the second set of 2 biopsies will be taken during the follow-up colonoscopy that is necessary in order to assess the presence of any residual or recurring dysplastic tissue
The risks associated with taking the biopsy include minor bleeding, and functional or mechanical changes after the procedure

There are also risks associated having blood withdrawn: pain at the injection site and bruising. The pain and bruising are self-limiting. The discomfort during the anthropometric and bio-impedance assessments and the liver ultrasound elastography are minimal.

Benefits for participating patients are related to receiving an intervention that may reduce risk factors for (the progression of) CRC and thereby improve their prognosis. The knowledge and healthier eating habits gained from the dietary counselling remain in the hands of the patients so they may experience continued benefits of following a MeD beyond the study period.

## Contacts

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## Trial sites

### Listed location countries

Netherlands

## Eligibility criteria

**Age**
Adults (18-64 years)
Elderly (65 years and older)

### Inclusion criteria

• Body mass index (BMI) > 25 kg/m2
• Age >18 years
• Presence of colorectal polyp > 2 cm
• Colorectal polyp eligible for endoscopic resection by means of ESD or EMR

### Exclusion criteria

• Not fluent in the Dutch language
• Currently follows a vegetarian or plant-based diet
• Is undergoing additional treatment (ie, additional surgery or chemoradiotherapy) due to being deemed as having high risk T1 CRC.
• Coagulation disorder
• Many, large diverticula
• Acute colitis

## Study design

### Design

**Study type:** Interventional

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| Masking: | Open (masking not used) |
| Control: | Uncontrolled |
| Primary purpose: | Prevention |

### Recruitment

NL

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|---|---|
| Recruitment status: | Recruiting |
| Start date (anticipated): | 16-01-2023 |
| Enrollment: | 25 |
| Type: | Actual |

## Ethics review

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| Approved WMO Date: | 15-08-2022 |
| Application type: | First submission |
| Review commission: | METC Leiden-Den Haag-Delft (Leiden)<br>metc-ldd@lumc.nl |

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| Approved WMO Date: | 01-05-2023 |
| Application type: | Amendment |
| Review commission: | METC Leiden-Den Haag-Delft (Leiden)<br>metc-ldd@lumc.nl |

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| Approved WMO Date: | 12-02-2024 |
| Application type: | Amendment |
| Review commission: | METC Leiden-Den Haag-Delft (Leiden)<br>metc-ldd@lumc.nl |

## Study registrations

### Followed up by the following (possibly more current) registration

No registrations found.

## Other (possibly less up-to-date) registrations in this register

No registrations found.

## In other registers

| Register | ID |
| --- | --- |
| CCMO | NL80524.058.22 |