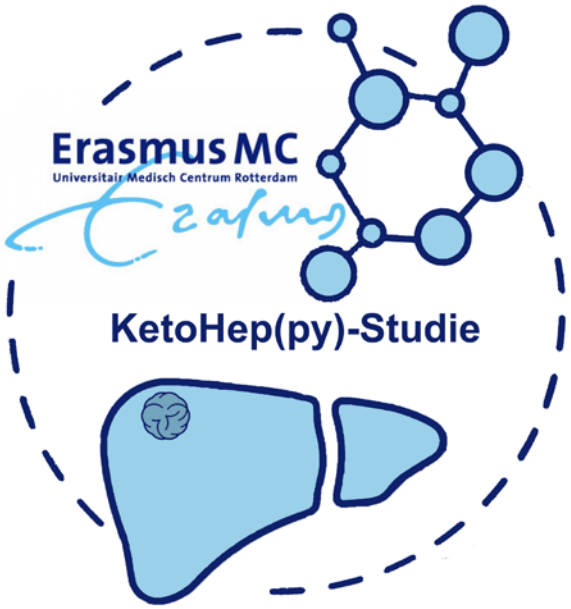
KETOgenic diet therapy in patients with HEPatocellular adenoma

Ketohep(py)-Study



RESEARCH PROTOCOL

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KETOgenic diet therapy in patients with HEPatocellular adenoma

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AR	Adverse Reaction
BDNF	Brain-Derived Neurotrophic Factor
BIA	Bioelectrical Impedance Analysis
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
DR	Dietary Restriction
EHR	Electronic Health Record
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HCA	HepatoCellular Adenoma
HDAC	Histone DeACetylase
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MRI	Magnetic Resonance Imaging
OC	Oral Contraceptives
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical

	company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAE	Trans-Arterial Embolization
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY
Rationale: Hepatocellular adenoma (HCA) is an uncommon, solid and benign liver lesion. Typically, it is a solitary lesion found in women within their reproductive years and it is strongly associated with the use of oral contraceptive medication (OC). Development of HCA is also associated with obesity and metabolic syndrome. More recently, studies have shown a rising incidence of HCA diagnosis, current prevalence is estimated to be between 0.001 and 0.004%. HCA consists off several subtypes: inflammatory, steatotic, β-catenin mutation associated and unspecified. A higher BMI is associated with inflammatory HCA, which is also associated with multiple lesions. Steatotic HCA more often consist of single lesions.

Management of HCA requires a multidisciplinary approach. For female patients, it depends on the associated symptoms, lesion size and location. All female patients are advised to stop using OC and maintain a healthy body weight. Women with HCA can be included in a surveillance period ("Wait-and-see"-strategy) for 6 months, after which a contrast-enhanced magnetic resonance imaging (MRI) is performed. The aim is to predict if larger lesions (>5cm) will regress, thus avoiding unnecessary surgery. Treatment modalities to further enhance the regression and avoid surgery are an interesting research possibility. As overweight is frequently observed in women with liver adenoma, metabolic changes are assumed to play a role and diet may help to reduce tumour size.

Dietary restriction, defined as reduced intake of food without malnutrition, may be effective. It's associated with metabolic changes, extended life span, lower risk of age associated diseases, improved fitness and increased resistance to acute stress. In combination with a ketogenic diet, it also reduces portal insulin concentrations, which down-regulate hepatic growth hormone receptors and reduces IGF-I synthesis. A recent study also shows the beneficial effect of eucaloric very-low-carbohydrate diet on disease control of acromegaly patients. Dietary restriction can be performed in different regimens such as short-term fasting or up to 30% reduced daily calorie intake. To explore the potential efficacy of dietary restriction, we aim to investigate whether the beneficial effects of a ketogenic diet with slight caloric restriction might increase the regression of HCA. This will further strengthen the treatment modality of close observation and avoid surgical resection.

Objective: to determine the effect of dietary restriction and the ketogenic diet on the regression of hepatocellular adenoma after 6 months.
Study design: A single-centre matched cohort study
Study population: Female subjects with a hepatocellular adenoma, who are entering a surveillance period including cessation of oral contraceptive medication and regular dietary advice.
Intervention: a ketogenic diet with +/- 30% less calories (approximately 35gr carbohydrate/ 1500 kcal per day) for 3 months, followed by a less strict ketogenic diet for 3 months (approximately 60gr carbohydrate/ 1500 kcal per day)

Main study parameters/endpoints: The difference in regression of the liver adenoma after 6 months compared to historical controls and the feasibility / adherence of the dietary intervention. Other secondary endpoints are Quality of Life, change in body weight, body composition, resting energy expenditure and plasma parameters.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The extent of the burden of our study is considered low. Dietary restriction and the ketogenic diet have been proven feasible and safe in previous studies. For this study, three extra blood samples by 3 venous punctures are taken. No extra visits to the hospital or imaging studies are needed in order to obtain all the information required for this study. Several standardized questionnaires are asked to be filled in during and after the diet. Mentioned questionnaires take about 10 minutes to complete. No other risks concerning the dietary intervention are to be expected.

1. INTRODUCTION AND RATIONALE

Hepatocellular adenoma (HCA), also known as hepatic adenoma, is an uncommon, solid, benign liver lesion (1-5). Typically, it is a solitary lesion found in young women within their reproductive years. HCA has been widely associated with the use of oestrogen-containing medication, f.i. contraceptive pills. The annual incidence of HCA in users of OCs has been estimated to be 30-40 cases per million OC users in comparison with one case per million non-users (1, 2). In several studies a consistent effect of sex-hormones in the development of HCA has been found. A 30-40 fold increase in the incidence has been confirmed in long term users of contraceptive pills (1, 6, 7). A dose-related risk ratio and regression after cancellation of use of contraceptive pills further confirmed the effect of sex-hormones in the development of HCA (4, 5, 8, 9). More recently, studies show a rising incidence of HCA diagnosis (10), current prevalence is estimated to be between 0.001 and 0.004% with an reported female: male ratio of 10:1 (11, 12). In large part this increasing incidence may be attributed to an increased discovery of incidental/asymptomatic HCA by extensive use of imaging.

HCA development is also associated with obesity, metabolic syndrome (10, 13-15) anabolic androgen use (16-18), rare genetic syndromes including glycogen storage diseases and familial adenomatous polyposis (3, 19, 20), especially in men (10, 13, 14). The mechanism causing the increased risk of developing HCA in metabolic syndrome has not been completely identified (13). HCA consists off several subtypes: inflammatory, steatotic, β-catenin mutation associated and unspecified (13, 15, 21). A higher BMI is associated with inflammatory HCA, which is also associated with multiple lesions (15). Steatotic HCA more often consist of single lesions (15).

A hepatocellular adenoma typically is detected by various ways. First, as an incidental finding on routine imaging on asymptomatic individuals. Secondly, as a symptomatic lesion presenting by pain in the right upper quadrant. Less often, the lesion may be detected because of acute haemorrhage resulting from rupture. This can be a life-threatening event if not identified on time (11, 21-24) and if the HCA ruptures into the abdominal cavity (3, 24). The risk of bleeding is increased if the HCA is larger than 5cm, use of hormones, pregnancy, exophytic morphology and an inflammatory subtype (24-26). A recent systematic review showed that the frequency of haemorrhage is 27,2% and a rupture with intraperitoneal bleeding was reported in 17,5% of patients (22, 24).

HCA is diagnosed by typical findings on ultrasound imaging and contrast-enhanced, cross-sectional imaging by MRI (22, 23, 27). For female patients a biopsy or fine-needle aspiration is not indicated (22, 23, 27). For male patients the diagnosis needs to be confirmed pathologically to exclude a malignant lesion (22, 23, 27).

Management of HCA requires a multidisciplinary approach. Men who are diagnosed with HCA are advised to undergo surgical resection. The rate of malignant transformation is significantly higher in males (47%) compared to females (4%) (11, 28). For female patients,

the management depends mainly on the associated symptoms, lesion size and location. All female patients are advised to stop using contraceptive medication and strive for a healthy body weight (4, 9, 22, 29, 30). Women with (a)symptomatic HCA can be subjected to a surveillance period for 6 months, after which a contrast-enhanced MRI is performed to check regression of the HCA. If the follow-up MRI shows a rapid regression or a lesion smaller than 5cm, further follow-up will be conducted (22, 23). Other treatment strategies are based on an internally validated model to predict regression (30). If the adenoma increases in size or shows heterogenous parts, surgical resection will be planned (22, 23). For a flowchart helpful in making decisions concerning the management of HCA, see figure 1.

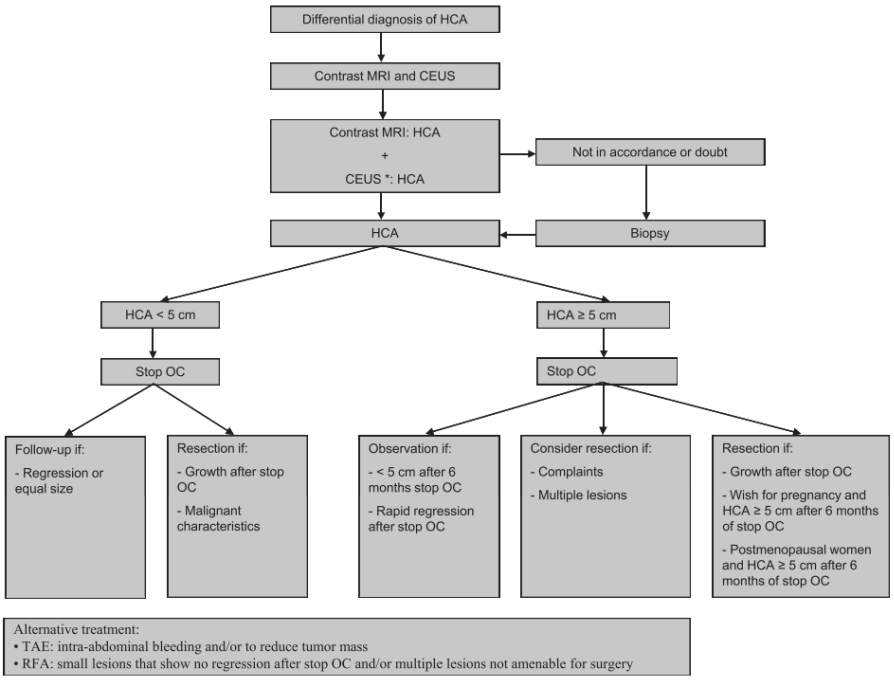

Figure 1: Flowchart helpful in deciding a management strategy for HCA, from van Aalten et al.

Management of hepatocellular adenoma in pregnant women or women with an active wish to conceive should require extra consideration. There may be an added risk of hormone-induced growth and therefore spontaneous haemorrhage (31-33). Currently, negative advice for pregnancy is only justified in women having tumours larger than 5 cm or those who have experienced complications of HCA in earlier pregnancies. In these cases, surgical resection before pregnancy should be considered (31-33). When a known asymptomatic HCA of <5cm is present, there is no evidence to advise against pregnancy (32, 33). For growing lesions or symptomatic lesions, treatment by surgical resection or trans-arterial embolization (TAE) is advocated before pregnancy.

Currently, treatment of (a)symptomatic HCA's focuses on a wait-and-see / active surveillance policy, aiming to reduce surgery. It consist of a 6-months observation period after which a MRI is performed (22, 23, 34). A model will be used to calculate a projected chance of regression in 1 or 2 years (30). Further treatment will be based on patient characteristics, size and regression of the HCA, projected chance of complete regression

and imaging characteristics of the HCA. Treatment modalities to further enhance regression are subject for research. Faster regression of the HCA could reduce patient's anxiety, waiting time, lead to a more reliable prediction of regression at one year and finally less surgical interventions.

Dietary / Caloric restriction (DR), meaning reduced intake of food without malnutrition, is associated with extended life span, lower risk of age associated diseases, improved fitness and increased resistance to acute stress (35-40). Caloric restriction can be performed in different regimens such as short-term fasting, or up to 30% reduced daily calorie intake for several weeks, or even indefinitely. Dietary restriction represents a non-invasive, non-expensive method of acquiring long term health benefits (40, 41). In combination with a ketogenic diet, it also reduces portal insulin concentrations, which down-regulate hepatic growth hormone receptors (42) and reduce IGF-I synthesis (43, 44). Caloric restriction and ketogenic diet also share biological responses implicated in metabolite-controlled longevity pathways, via production of ketone bodies. Production of ketone bodies such as b-hydroxybutyrate from fatty-acid catabolism may operate as endogenous histone deacetylase (HDAC) inhibitors and may contribute to epigenetic control of gene expression, DNA repair, and genome stability (45). Ketogenesis also promotes synaptic plasticity and neurogenesis by increasing the expression of brain-derived neurotrophic factor (BDNF) (45). Dietary restriction and/or fasting further increases expression of cytoprotective genes, increases immunomodulation via increased anti-inflammatory cytokine production and also decreases the expression of inflammatory markers (46-48). So far, the benefits of fasting and/or dietary restriction in rodents (46, 47, 49-51) have partly been translated to humans. DR has been proven feasible and safe in well-nourished patients who are opting for surgery (52-54).

The classic ketogenic diet is a high-fat, adequate-protein, low-carbohydrate diet that produces metabolic changes associated with the starvation state (55-58). Changes in plasma ketones, insulin, glucose, glucagon, and free fatty acids can occur within hours of starting the diet and can be profound (55). A recent study also shows the beneficial effect of eucaloric very-low-carbohydrate ketogenic diet on disease control of acromegaly patients patients via lowering of IGF-1 levels (59). In a recent randomized controlled trial, patients with type 2 diabetes improved their glycaemic control and lost more weight after being randomized to a very low-carbohydrate ketogenic diet and lifestyle online program rather than a conventional, low-fat diabetes diet online program (57). Potential side effects of the ketogenic diet include constipation, diarrhoea, nausea, vomiting, and very rarely pancreatitis (60-62). The side effects can lessen with continued diet use and minor adjustments under supervision of a dietician. Pharmaceutical intervention or diet discontinuation is rarely indicated.

To further strengthen the "the wait-and-see / active surveillance" strategy as a successful treatment modality of HCA we aim to investigate whether the beneficial effects of a ketogenic diet with moderate caloric restriction might increase the rate of regression of HCA.

2. OBJECTIVES

Primary Objective: to determine the effect of a moderate calorie restricted, ketogenic diet on the reduction in size of hepatocellular adenoma after 6 months in adult female patients.

Secondary Objectives:

- To determine the feasibility / adherence to the ketogenic diet
- To identify predictors of the primary endpoint
- To assess possible change in the estimated chance of HCA regression to 0,5 cm at 1-2 years due to the ketogenic diet, estimated by prediction model.
- To determine the change in liver fat content
- To investigate potential side effects & burden of diet
- The determine the effect on Quality of Life (QoL)
- To assess change in body weight and plasma parameters
- To investigate the effect of the diet on change in body composition and resting energy expenditure

3. STUDY DESIGN

We aim to answer our research questions by conducting a single-centre, proof of concept interventional matched cohort study.

Main study population

Our newly acquired cohort will be compared with a matched historical control group acquired during an earlier study (30). This large multicentre retrospective cohort developed a prediction model for patients with HCA (N=180, HCA diameter at baseline: 82.0mm (65-100) and follow-up: 65.0mm (56-80) (Median (IQR)). This prediction model estimates the probability of HCA regression to <5cm at 1 and 2 years follow-up (30) with an internally validated c-index of 0.79.

The prediction model the authors developed was translated into a chance assessment tool. Included predictors in this model are: diameter at diagnosis, diameter at first follow-up, dates of diagnosis and first follow-up and HCA-subtype. This chance assessment tool will provide the estimated chance of regression to 0,5 cm at 1-2 years after diagnosis and is currently used in clinical practice at Erasmus University Medical Center. The chance tool is available via https://hcaprediction.shinyapps.io/calculator/. This prediction model will also be used for our included patients, as part of standard of care.

Matching to the earlier acquired patient cohort will be based on subject age, use of oral contraceptives, ethnicity, weight, type of HCA, size of HCA and number of lesions at baseline. Matching will be conducted via optimal nearest-neighbor matching on propensity score, in a 1:1 ratio without replacement (63). Examination of the balance on covariates resulting from the matching method will be conducted to determine if matching has been successful.

Study subpopulation

As a nested pilot-study we aim to acquire a small sub-cohort of subjects with a slightly different population base criterium, see chapter 4.1. The inclusion and exclusion criteria will be applied in the same way as in the main study population.

This sub-cohort of subjects will be following study procedures according to the procedure defined for the main study population, as stated in chapter 8. They will also be compared with a matched historical control group, in exactly the same way as the main study population. As we expect that the difference in the population base criterium will be a significant confounder in statistical analysis, analysis of this subgroup will be conducted separately, but will be done according to the procedure defined for the main study population, as stated in chapter 10.

4. STUDY POPULATION

4.1 Population base

Female subjects with a hepatocellular adenoma of any size, who are receiving consultation at the outpatient clinic of Erasmus MC University Medical Centre in Rotterdam, the Netherlands.

Main study population
Female subjects with a hepatocellular adenoma, who are advised to enter an initial surveillance period of 6 months after diagnosis, after reviewing their case by the multidisciplinary team of experts, including a hepatobiliary surgeon, a hepatologist and a liver radiologist.

Study subpopulation
Female subjects with a hepatocellular adenoma, who after the initial surveillance period of 6 months including the regular treatment advice of losing weight and cessation of use of oral contraceptives, did not or only minimally (5mm) experience a reduction of the size of the HCA. Standard treatment for this subgroup after the 6 months of initial surveillance would consist of an additional surveillance period of 6 months, after which another MRI would be performed to assess the change in size of HCA.

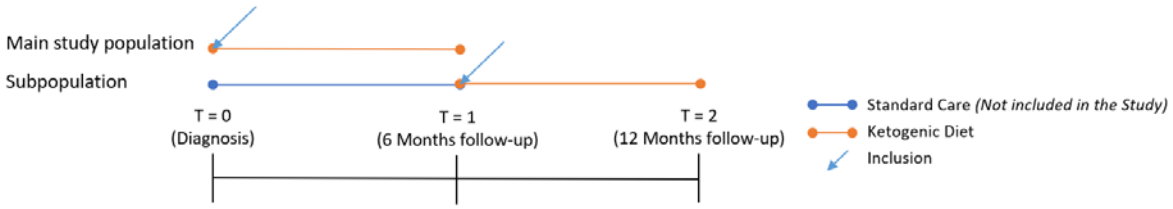

Figure 2: Graphical representation of main study population and pilot subpopulation

4.2 Inclusion criteria

- Age 18-50 years
- BMI > 25 kg/m2
- Provide written consent

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Current pregnancy or breastfeeding
- Diabetes Mellitus type 1 or 2
- Insufficient understanding of the Dutch language
- Participation in another clinical study

4.4 Sample size calculation

We based our sample size calculations on the large multicentre retrospective cohort mentioned earlier study (30). This study calculated a median (IQR) HCA diameter at baseline of 82.0mm (65-100) and at 6-month follow-up of 65.0mm (56-80).

Based on this, considering a relevant effect of 20% difference in mean tumour size after 6 months, we estimated an average additional decrease of 13mm in the intervention group when compared to our earlier collected (30).

With a two-sided alpha set at 0.05, power at 0.95, we calculated that at least 51 subjects are required. Anticipating +/- 10% drop-out, we aim to include 55 participants in this study. We aim to include all study subjects in 1 – 1,5 years.

Since the subpopulation is a pilot study, we did not perform a sample size calculation. For this subpopulation, we hypothesized a sample size of 20 subjects. We estimated that this sample size is large enough to provide a reliable initial answer.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

All subjects will receive a diet for 6 months (see table 1 & 2 for the example diet). In this ketogenic diet the amount of carbohydrates is restricted, the first three months to approximately 35 grams per day, the second part for month 4-6 to approximately 60 grams per day. This amount of carbohydrates will lead to ketogenesis. Emphasis is placed on the use of products high in polyunsaturated and monounsaturated fatty acids (diet margarines, oils, fish, nuts), preferably with vegetable and marine sources of protein, in concordance with the national nutritional guidelines (64). We aim to moderately reduce the calories to about 70-80% of the daily energy requirements.

After the study period of 6 months, participants are free to choose which diet they want to follow. They can choose to keep following the moderate carbohydrate restricted diet, or switch to a diet according to the national nutrition guidelines. If they would like advice from a dietician, they can be referred to a dietician via their family doctor.

After participating in this study, subjects will receive further treatment according to standard clinical practice: they will be included in regular follow-up or undergo treatment for their HCA. The frequency of follow-up or possible invasive treatment depends on the findings on the MRI made after six months and the calculated chance of regression, according to current protocol.

Diet Overview:
Month 1-3: Low carbohydrate, ketogenic diet containing approximately 1500 kcal, 35 grams of carbohydrate and 0.8 g/kg of protein /day
Month 4-6: Moderate carbohydrate, ketogenic diet containing approximately 1500 kcal, 60 grams of carbohydrate and 0.8 g/kg of protein /day

5.2 Use of co-intervention

Not applicable.

5.3 Escape medication

Not applicable.

Table 1 Example menu month 1-3
Koolhydraatbeperkt (35 g/dag) & energiebeperkt (1500 kcal, adequaat eiwit (0.8 g E/kg)

Gemiddeld gewicht (80 kg)		**Energie (kcal)**	**Eiwit totaal (g)**	**Vet totaal (g)**	**Koolhydraten totaal (g)**
Ontbijt					
Brood, koolhydraatarm Of vervanging zie variatielijst	1 snee (= 35g)	84	6,0	3,1	5,0
Margarineproduct 60% vet <17 g verz vetz ongez	5g	27	0,0	3,0	0,0
48+ kaas of vleeswaar, stukje vis of 0,5 gekookt ei	1 voor 1 snee (= 20g)	74	4,6	6,1	0,0
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		185	10,6	12,2	**5,0**
In de loop van de ochtend					
Aardbeien of ander fruit	50g	14	0,3	0,0	2,5
Thee/koffie zonder suiker	2 bekers (= 500ml)	0	0,0	0,0	0,0
Subtotaal		14	0,3	0,0	**2,5**
Middagmaaltijd					
Brood, koolhydraatarm of vervanging zie variatielijst	1 snee (= 35g)	84	6,0	3,1	5,0
Margarineproduct 60% vet <17 g verz vetz ongez	5g	27	0,0	3,0	0,0
Maaltijdsalade van IJsbergsla	1,5 schaaltje (= 70g)	10	0,6	0,2	1,1
Zalm, gerookt of ander vis	25g	46	5,5	2,7	0,0
Cherrytomaatje, rauw	4 stuks (= 40g)	12	0,4	0,3	1,6
Komkommer zonder schil	25g	3	0,1	0,1	0,5
Avocado	50g	100	0,9	9,8	0,9
Dressing van: Olie, alle soorten	1,5 eetlepel (= 15g)	134	0,0	14,9	0,0
Mayonaise	0,5 eetlepel (= 10g)	66	0,1	7,2	0,4
Subtotaal		482	13,6	41,3	**9,5**
In de loop van de middag:					
Noten, gemengd, ongezouten	1 handje (= 25g)	162	5,3	13,8	3,4
Thee/koffie zonder suiker of suikervrije frisdrank	2 bekers(=500ml)	0	0	0	0
Avondmaaltijd					
Aardappelen, gekookt	28g	24	0,5	0,1	5,0
Groente, gekookt	140g	43	2,7	0,7	5,0
Kipfilet,vlees of vis, onbereid	1 stuks (= 125g)	136	29,1	2,3	0,0
Bereiden in Olie, alle soorten	1,5 eetlepel (= 15g)	134	0,0	14,9	0,0
Margarine, vloeibaar, ongezouten	0,5 eetlepel (= 5g)	37	0,0	4,1	0,0
Subtotaal		374	32,3	22,1	**10,0**
In de loop van de avond					
Griekse yoghurt, vol	125g	156	5,9	12,5	4,5
Olie, alle soorten Bijvoorkeur kokosolie	1,5 eetlepel (= 15g)	134	0,0	14,9	0,0
Thee/koffie zonder suiker /water of sv frisdrank	2 bekers (500ml)	0	0	0	0
Subtotaal		290	5,9	27,4	4,5
Totaal	**2000ml**	**1507**	**68**	**116,8**	**34,9**

Table 2 Example menu month 4-6.
Koolhydraatbeperkt (60 g/dag) & energiebeperkt (1500 kcal, adequaat eiwit (0.8 g E/kg)

Gemiddeld gewicht (80 kg)		**Energie (kcal)**	**Eiwit totaal (g)**	**Vet totaal (g)**	**Koolhydraten totaal (g)**
Ontbijt					
Brood, koolhydraatarm Of vervanging zie variatielijst	2 sneden (=70g)	169	12,0	6,2	10,0
Margarineproduct 60% vet <17 g verz vetz ongez	2 x 5 gram	11	0,0	1,2	0,0
48+ kaas	1 voor 1 snee (=20g)	74	4,6	6,1	0,0
Vleeswaren, gemiddeld Vis of ei	1 voor 1 snee (=20g)	35	2,4	2,7	0,4
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		289	19,0	16,2	**10,4**
Ochtend					
1 stuks Fruit (geen banaan of druiven)	150 g	74	1,2	0,0	16,5
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		74	1,2	0,0	16,5
Middagmaaltijd					
Brood, koolhydraatarm Of vervanging zie variatielijst	2 sneden (=70g)	169	12,0	6,2	10,0
Margarineproduct 60% vet <17 g verz vetz ongez	2 x 5 gram	11	0,0	1,2	0,0
48+ kaas	1 voor 1 snee (=20g)	74	4,6	6,1	0,0
Vleeswaren, gemiddeld Vis of ei	1 voor 1 snee (=20g)	35	2,4	2,7	0,4
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		289	19,0	16,2	**10,4**
Middag					
Noten, gemengd, ongezouten	1 handje (=25g)	162	5,3	13,8	3,4
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		162	5,3	13,8	3,4
Avondmaaltijd					
Aardappelen, gekookt	1 klein (=50g)	43	0,9	0,2	8,9
Groente, gekookt	3 opscheplepels (= 150 g)	46	2,9	0,8	5,4
Kipfilet, vis of vlees onbereid	1 stuks (=125g)	136	29,1	2,3	0,0
Olie, alle soorten	1,5 eetlepel (=15g)	134	0,0	14,9	0,0
Margarine vloeibaar 80% vet	0,5 eetlepel (=5g)	37	0,0	4,1	0,0
Subtotaal		396	32,9	22,3	**14,3**
In de loop van de avond					
Griekse yoghurt, vol Hieraan toevoegen:	125 g	156	5,9	12,5	4,5
Olie, alle soorten Bij voorkeur kokosolie	1,5 eetlepel (=15g)	134	0,0	14,9	0,0
Thee zonder suiker	2 bekers (=500g)	0	0,0	0,0	0,0
Subtotaal		290	5,9	27,4	4,5
Totaal	**2000 ml**	**1500**	**83,3**	**95,9**	**60,0**

6. INVESTIGATIONAL PRODUCT

Not applicable, this study does not involve an investigational product.

6.1 Name and description of investigational product(s)

Not applicable, this study does not involve an investigational product.

6.2 Summary of findings from non-clinical studies

Not applicable, this study does not involve an investigational product.

6.3 Summary of findings from clinical studies

Not applicable, this study does not involve an investigational product.

6.4 Summary of known and potential risks and benefits

Not applicable, this study does not involve an investigational product.

6.5 Description and justification of route of administration and dosage

Not applicable, this study does not involve an investigational product.

6.6 Dosages, dosage modifications and method of administration

Not applicable, this study does not involve an investigational product.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable, this study does not involve an investigational product.

6.8 Drug accountability

Not applicable, this study does not involve an investigational product.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable, this study does not involve a non-investigational product.

7.1 Name and description of non-investigational product(s

Not applicable, this study does not involve a non-investigational product.

7.2 Summary of findings from non-clinical studies

Not applicable, this study does not involve a non-investigational product.

7.3 Summary of findings from clinical studies

Not applicable, this study does not involve a non-investigational product.

7.4 Summary of known and potential risks and benefits

Not applicable, this study does not involve a non-investigational product.

7.5 Description and justification of route of administration and dosage

Not applicable, this study does not involve a non-investigational product.

7.6 Dosages, dosage modifications and method of administration

Not applicable, this study does not involve a non-investigational product.

7.7 Preparation and labelling of Non-Investigational Medicinal Product

Not applicable, this study does not involve a non-investigational product.

7.8 Drug accountability

Not applicable, this study does not involve a non-investigational product.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Our main study endpoint is the difference in tumour regression. The size (largest diameter in cm) of the largest hepatocellular adenoma found on MRI will be measured at T_0 = time of inclusion and at T_5= 6 months after start of the intervention. Mean regression will be calculated after which it will be compared to the regression in tumour size of our (earlier acquired) cohort and the subsequent internally validated model published earlier (30).

8.1.2 Secondary study parameters/endpoints

- Feasibility / Adherence to the ketogenic diet
    - Self-reported adherence, measured via in-house used questionnaire “Ketodieet”. Questionnaire has not validated but partly based on “Vragenlijst Intensive Monitoring Lareb (65)”, measures the amount of days subjects did not use the ketogenic diet. Serves as a tool during outpatient clinic visits to the dietician.
    - Ketone levels in urine, self-measured thrice weekly by urine strips (Siemens, Multi stix 10SG)
    - Ketone levels, measured at physical meetings with dietician, by Ketosemeter CareSense Dual
- Side effects & burden of diet, measured via in-house used questionnaire “Ketodieet”. Questionnaire has not validated but partly based on “Vragenlijst Intensive Monitoring Lareb (65)”, measures possible side effects mentioned by subjects during the use of the ketogenic diet. Serves as a tool during outpatient clinic visits to the dietician.
- Results of the prediction model/chance assessment tool (30), providing the estimated chance (%) of HCA regression to 0,5 cm at 1 and 2 years after diagnosis.
- Change in Liver fat content, measured on MRI
- Change in plasma parameters:
  Fasting glucose, fasting insulin, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, free fatty acids, triglycerides levels, branched amino acids, IGF -1, Growth hormone (hGH), β-Hydroxybutyrate
- Quality of life measured by RAND-36 (66-69), Dutch version 2, 2012. Higher score meaning higher QoL
- Change in body weight (kg)
- Change in body composition (fat mass / fat free mass)
    - via Bio-electrical Impedance Analysis (Bodystat quadscan 4000, Euromedix, Leuven, Belgium)
- Resting Energy Expenditure
    - Via Quark RMR, Cosmed Benelux B.V., Nieuwegein, The Netherlands

8.1.3 Other study parameters

- Baseline subject characteristics such as age, sex, weight, length, BMI, oral contraceptive use, other medication use, comorbidities, size of hepatocellular adenoma at baseline, multiple or single lesion, amount of hepatocellular adenoma's at baseline, subtype of hepatocellular adenoma, time since initial diagnosis, time since last imaging study, imaging study provided (MRI/CT/Ultrasound), referring hospital and active pregnancy wish.
- Macronutrient intake (3-day food diary)
- Physical activity by IPAQ questionnaire (70, 71), Dutch version 1, 23-01-2011. Higher score means a higher level of physical activity
- Incidence of symptomatic HCA, defined as:
    - HCA rupture/bleeding confirmed on CT / MRI
    - for which admittance into the hospital or (endovascular) treatment is indicated.

8.2 Randomisation, blinding and treatment allocation

All subjects in our study will be allocated to the dietary intervention and henceforth cannot be blinded.

8.3 Study procedures

Standard procedure / current best clinical practice

Main study population

Subjects present themselves at our outpatient clinic after referral from a peripheral or other tertiary/university hospital. Subjects will be receiving additional imaging studies (f.i. an MRI) if the necessary imaging study has not been conducted at the peripheral/referring hospital, according to current standard protocol/clinical practice. Afterwards their case will be discussed in our multidisciplinary meeting, including a (liver) radiologist, hepatobiliary surgeon, gastroenterologist / hepatologist and dietician.

During current clinical practice, subjects will enter the "Wait and See" / "Active Surveillance" period for 6 months (30) based on several criteria. See figure 1 for the management flowchart.

Subpopulation

Subjects eligible for inclusion into the study subpopulation are already being treated via the outpatient clinic via active surveillance. After the initial 6 months of active surveillance (with advice consisting of cessation of use of oral contraceptives and aiming for weight loss), subjects have been subjected to an MRI. If this MRI shows no or only a minimal (5mm) reduction of the size of the HCA, patients are subjected to another active surveillance period of 6 months after which another MRI is performed. We want to provide the patients with the option to adhere to the ketogenic diet as a potential therapeutic option.

Intervention / Study procedure

Time of inclusion:

Eligible subjects visiting the outpatient clinic after discussion of their case in the multidisciplinary meeting are invited to participate in the study. The possible participant is asked whether she wants to be approached for further information on this study. The gastro-enterologist / hepato-biliary surgeon shortly explains the purpose of the study after which, if the subject agrees to it, the study investigator explains the study design and purpose in more detail. Subjects receive written information and are offered a reflection period of one week before they are contacted again about participation in the study. In case informed consent is given, subjects will receive an appointment with a dietician.

Follow-up and outpatient visits will be identical to normal procedure, except the extra appointments with the dietician and the additional blood samples drawn by venous puncture on T_0, T_3 and T_5.

Consultation by Dietician

This consult will focus on explaining the study diet, nutritional assessment, adherence to the diet, measurements, advice and instruction in case of possible side effects.

Nutritional assessment

Nutritional assessment will take place during the study visits at the outpatient clinic and consists of:

- Resting energy expenditure (Indirect calorimetry)
- Body composition by bioelectrical impedance analysis (BIA)
- 3-day food diary filled in at home and cross checked during the study visits.
- Anthropometric measurements, body weight will be measured during visits to the outpatient clinic.

Nutritional assessment is part of standard dietetic care. For the operational procedures we refer to the NAP website (72). The procedure involves measurement of inspired and expired air. Participants are asked to lay down in a supine position for approximately half an hour and to breath in and out of a ventilated hood. Bio impedance involves the patient to lay down in a supine position or stand on a special scale with special handgrips for 1 minute.

Questionnaires

Questionnaires will focus on adherence and any possible side effects / symptoms subjects might experience during the ketogenic diet. Other questionnaires conducted during study focus on quality of life and physical activity.

Consultation by Clinician / Investigator

This consult will focus on any possible abdominal pain / complaints, any complaints after halting oral contraceptives, advice to currently avoid pregnancy and advice concerning when to contact our outpatient clinic.

Imaging studies

Subjects will receive their follow-up MRI according to normal treatment protocol after 6 months. No additional imaging studies will be performed for this study, extra measurements will be made on already acquired imaging studies to calculate the liver fat content.

Laboratory testing

Blood will be acquired by venous puncture for measuring fasting parameters. Blood sampling will be done thrice, each time 2 tubes of 10ml will be withdrawn.

Urine testing

Ketones / Acetoacetate in urine will be assessed thrice weekly by subjects themselves using a simple urinestrip to determine / confirm ketose state and therefore adherence. Subjects will be carefully instructed during their clinical appointments on how to use these strips.

Followup overview:

Table 2: Study overview, window for appointments: T_1&T_2: +/- 3 days, T_3-T_5: +/- 1 week

Procedure	T_0 Baseline	T_1 2 weeks	T_2 6 weeks	T_3 3 months	T_4 4 months	T_5 6 months
Dietary restriction	Start diet (Phase 1)			Start diet (Phase 2)		End of diet
Informed Consent	X					
Type of contact	Physical	Telephone	Telephone	Physical	Telephone	Physical
Consultation by Dietician	Outpatient clinic	By phone	By phone	Outpatient clinic	By phone	Outpatient clinic
Consultation by Clinician / investigator	Outpatient clinic					Outpatient clinic
MRI-Followup						X
Laboratory testing	X			X		X
Anthropometric measurements	X			X		X
Nutritional assessment:	X			X		X
Questionnaires:						
IPAQ:	X			X		X
Rand-36:	X			X		X
Food Diary:	X	X	X	X	X	X
Side effects:		X	X	X	X	X

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

Inclusion takes place prior to starting the diet. When subjects develop severe symptoms of their hepatocellular adenoma, f.i. haemorrhage, they will be withdrawn from the study and they will be treated according to the current best clinical practice, f.i. by trans-arterial embolization. Severe symptoms are defined as symptoms for which hospital admittance is indicated / invasive treatment is indicated. Subjects will also be withdrawn from the study if they become pregnant.

8.5 Replacement of individual subjects after withdrawal

When a subject is withdrawn from the study before starting the ketogenic diet or if they opt out themselves for any reason, a new subject will be included in our study.

8.6 Follow-up of subjects withdrawn from treatment

Subjects will be still be included in follow-up if they did not complete their dietary intervention.

8.7 Premature termination of the study

There are no projected risks to the safety and feasibility of this trial, see chapter 13. Therefore, no criteria for premature termination were formed.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor, investigator or principal investigator will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to ketogenic diet. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. When adverse or serious adverse events are observed, the principal investigator will be informed. Subsequently, the principal investigator takes responsibility for the correct processing of the events.

Known minor side effects of the ketogenic diets (constipation, diarrhoea, nausea and/or vomiting) will be analysed separately and are therefore not considered an adverse effect.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that
- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable since this study does not involve an investigational medicinal product.

9.3 Annual safety report

Not applicable since this study does not involve an investigational medicinal product.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

The assessment of a Data Safety Monitoring Board has been taken into consideration by the involved investigators of this study. There has been chosen not to establish a DSMB for the following reasons:

- The interventional product used in this study is not a medicinal product.
- The nutritional intervention will be given under strict supervision of a dietician
- Subjects included in this study are otherwise healthy, mentally capable, non-critically-ill subjects and are not incapacitated.
- The interventional diet used in this study is not known to harm the involved subjects.

10. STATISTICAL ANALYSIS

Categorical data will be presented as numbers (percentage) and continuous variables as mean (standard deviation/normal distribution) or median (interquartile range/no normal distribution).

An Intention-to-treat analysis will be conducted to account for the feasibility of the caloric restricted and ketogenic diet. A two-sided significance level of 0.05 will be used for all primary and secondary analyses unless stated otherwise.

Statistics will be computed using IBM SPSS software version 21.0 (Chicago, IL) or R version 4.0.2 or newer.

10.1 Primary study parameter

The primary endpoint is the reduction in size (in cm) of the largest diameter of the largest hepatocellular adenoma after 6 months, compared to our matched historical controls. A multivariable linear regression model will be used to estimate the difference in tumour regression between the treatment group and the historical control group. The model will be adjusted for age, change in body weight, the ketogenic diet and oral contraceptive use at baseline.

Subpopulation: the same model will be constructed but will not be adjusted for oral contraceptive use at baseline.

10.2 Secondary study parameters

In the secondary analysis, the multivariable linear regression model analysis from the primary analysis will be used to identify independent variables (age, change in body weight, subjected to ketogenic diet and oral contraceptive use at baseline) as predictors of the primary endpoint. An additional analysis will be performed including adherence to the diet as independent variable.

Analysis for adherence to the ketogenic diet will be done via descriptive statistics. Adherence to the diet will be calculated as a percentage.

Side effects of the ketogenic diet will be analyzed via descriptive statistics. Occurrence of side effects will be given as a percentage.

Results of the prediction model/chance assessment tool (30), providing the estimated chance (%) of HCA regression to 0,5 cm at 1 and 2 years after diagnosis will be compared between our intervention group and the matched historical controls by the Chi-square test.

Change in liver fat content (%) will be compared in our intervention group by a multivariable linear regression model. The independent variables in this model will include age, change in body weight and oral contraceptive use at baseline. An additional analysis will be performed including adherence to the diet as independent variable.

Changes in plasma parameters will be compared in our intervention group by using descriptive statistics, t-test in the case of continuous outcome variables with normal distribution or non-parametric test in the case of non-normality. The Chi-square test will be used for categorical data.

Change in quality of life will be compared over time in our intervention group by a mixed-effects model analysis, taking repeated measurements into account. The independent variables in this model will include age and change in body weight. An additional analysis will be performed including adherence to the diet as independent variable.

Mean change in body weight, change in body composition and resting energy expenditure will be estimated in our intervention group by a mixed-effects model analysis, taking repeated measurements into account. The independent variables in this model will include age and sex. An additional analysis will be performed including adherence to the diet as independent variable.

Subpopulation: the same models will be constructed, but the models will not be adjusted for oral contraceptive use at baseline. This subgroup will also have no results of the prediction model/chance assessment tool and therefore this cannot be computed in the subpopulation.

10.3 Other study parameters

General patient characteristics at baseline will be compared between subjects and the matched historical control group using descriptive statistics.

Change in physical activity and resting energy expenditure will be compared in our intervention group by a mixed-effects model analysis, taking repeated measurements into account. The independent variables in this model will include age, sex and compliance to the diet.

A survival analysis will be performed of symptomatic HCA, defined as rupture / bleeding confirmed on CT / MRI for which admittance into the hospital and/or (endovascular) treatment is indicated.

10.4 Interim analysis

No interim analysis will be performed.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (version 9, 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Participants will be recruited for the trial during their second visit to our outpatient clinic after their referral for additional imaging (on indication) and management of their incidental hepatocellular adenoma. The gastro-enterologist or hepatobiliary surgeon informs the subject about the study procedures and the general outline of the trial. If the subject is interested to be included in the study after the short explanation of their physician, she will be referred to the study investigator or dietician for further information concerning the study design and purpose. Subjects receive written information and are offered a reflection period of one week. It is the responsibility of the investigators to provide the subjects with detailed information, both orally and in writing, about the aims and design of the study, as well as the study procedures involved. The subjects will have the opportunity to ask all possible questions and receive additional information throughout enrolment in this study. Possible participants will be asked to make their decision 1 week after the visit to our outpatient clinic.

11.3 Objection by minors or incapacitated subjects

Not applicable, as minors or incapacitated subjects are not involved in this study.

11.4 Benefits and risks assessment, group relatedness

Based on the hypothesis and earlier studies that long-term caloric restriction and the ketogenic diet induce beneficial effects, the diet intervention group may experience better subjective wellbeing and weight loss compared to those in the control group. Potential side-effects can be managed by the dietician involved. This study aims to investigate the merits of caloric restriction and the ketogenic diet in the treatment of hepatocellular adenoma.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study:

- € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
- € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

There will be no compensation for participation in this study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be stored in Castor, OpenClinica or a similar program that complies with laws and regulations and will be handled confidentially. Upon inclusion into this study, each subject will be assigned a study number (KETO-001, KETO-002, etc). This study number will be listed on all study related documentation. In this study, no personal documents will be listed. The investigator will keep a subject identification log that contains the key to the code, i.e. a record of the personal identification data linked to each subject study number. This record is filed at the investigational site and can only be accessed by the investigator and the supporting site staff. Research data that can be traced to individual persons can only be viewed by authorized personnel. These persons are the members of the research team, members of the healthcare inspection and monitors. Data will be saved for a maximum of 15 years after completion of the study. All data will be handled confidentially in compliance with the EU General Data Protection Regulation (in Dutch: Uitvoeringswet ‘Algemene Verordening Gegevensbescherming’ UAVG)).

In the informed consent, subjects are asked whether or not the data and documents collected for this study may be used for further research. When subjects sign this separate consent, (future) investigators in need for the information are able to access these data.

12.2 Monitoring and Quality Assurance

Source data will be obtained from the electronic health record (EHR) and completed questionnaires. The result of our risk classification assessment is ‘research with negligible risk’. As described by the Erasmus MC METC monitoring plan (version 19th of February 2013), we will have a monitoring frequency of once per year that will be performed as recorded in the monitoring plan document appendix A.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial;
- the quality or safety of any intervention used in the trial.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last subject last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

The principal investigator is responsible for the public disclosure and publication of the research data. The protocol and the (final) results of the study will be summarized in a report / article and will be submitted for publication in a medical journal. Also, all participating subjects or their family will receive a layman's summary of the (final) results of the study, if they so desire.

The trial will be registered in a public trial registration registry after approval from the METC (in Dutch: Nederlands Trial Register (NTR), available by: http://www.trialregister.nl/trialreg/index.asp).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Chapter 13.1 is skipped, because in this trial no medicinal products will be used in the intervention. Therefore, in this trial no extra risk is associated with the medicinal products.

a. Level of knowledge about mechanism of action
Not applicable

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
Not applicable

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?
Not applicable

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Not applicable

e. Analysis of potential effect
Not applicable

f. Pharmacokinetic considerations
Not applicable

g. Study population
Not applicable

h. Interaction with other products
Not applicable

i. Predictability of effect
Not applicable

j. Can effects be managed?
Not applicable

13.2 Synthesis

Risk associated with observation of HCA

Follow up / conservative treatment of (a)symptomatic hepatocellular adenoma in women of fertile age has been proven feasible and safe and is current best clinical practice (22, 23, 30, 34). Especially asymptomatic adenoma's <5cm have an extremely low chance of rupture (25, 26) and are treated almost exclusively by careful follow-up.

Adenomas larger than 5cm in subjects who are using oral contraceptives at the time of diagnosis also have a significant chance of an excellent response on conservative therapy (22, 23, 30, 34). Subjects will receive ample instructions and information considering the

nature of the disease and will by carefully instructed when to contact the hospital and/or their general practitioner in the case of clinically relevant symptoms.

Conservative treatment consisting of discontinuing oral contraceptives and weight loss is a low-risk treatment of incidental hepatocellular adenoma and continuing this practice in the current study does not increase the risk of clinically significant complications.

Risk associated with the ketogenic diet

Study participants will be in close contact with the dieticians / study investigators when participating in the study/following the diet. In case of emergencies, countermeasures including discontinuation of the diet and appropriate (supportive) treatment as dictated by the subject's clinical status. Subjects will receive extensive and practical information concerning the diet.

Potential side effects of the ketogenic diet consist of constipation, diarrhea, nausea, vomiting, and rarely pancreatitis (60). Supervision of a qualified dietician is standard in our study and provides close observation to the amount of side effects. The side effects can improve with continued diet use and with minor adjustments under supervision of a dietician. Strategies employed by our dieticians are recommending multiple small meals throughout the day, increasing fibre, sodium, and fluid intake and daily exercise.

Administering a ketogenic diet did not produce any significant side effects for a longer period of time (60-62). Therefore, we expect it is safe to use a ketogenic diet for our subjects and the risks associated with participation can be considered negligible. Therefore, in our opinion the remaining risks are acceptable for the subjects to participate in the study.

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