

Doubling diet fat on sugar ratio in children with mitochondrial OXPHOS disorders: Effects of a randomized trial on resting energy expenditure, diet induced thermogenesis and body composition

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► **To cite this version:**

Laurent Beghin, Stephanie Coopman, Manuel Schiff, Joseph Vamecq, Karine Mention-Mulliez, et al.. Doubling diet fat on sugar ratio in children with mitochondrial OXPHOS disorders: Effects of a randomized trial on resting energy expenditure, diet induced thermogenesis and body composition. Clinical Nutrition, Elsevier, 2016, 35 (6), pp.1414-1422. 10.1016/j.clnu.2016.03.015 . hal-02177005

HAL Id: hal-02177005

<https://hal.univ-lille.fr/hal-02177005>

Submitted on 8 Jul 2019

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1 **Doubling diet fat on sugar ratio in children with mitochondrial**
2 **OXPHOS disorders: Effects of a randomized trial on resting energy**
3 **expenditure, diet induced thermogenesis and body composition**
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24 **Short title:** Doubling diet fat on sugar ratio in mitochondriopathies

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36 *Abbreviations:*

37 ANOVA : Analysis Of Variance.

38 BD : Basal Diet

39 CD : Challenging Diet

40 CONSORT : Consolidated Standards of Reporting Trials

41 DIT : Diet Induced Thermogenesis

42 MOD : Mitochondrial OXPHOS Disorder

43 OXPHOS : Oxidative Phosphorylation

44 REE : Resting Energy Expenditure

SUMMARY

45 *Background and aims:* Mitochondrial OXPHOS disorders (MODs) affect one or several complexes
46 of respiratory chain oxidative phosphorylation. An increased fat/low-carbohydrate ratio of the diet
47 was recommended for treating MODs without, however, evaluating its potential benefits through
48 changes in the respective contributions of cell pathways (glycolysis, fatty acid oxidation) initiating
49 energy production. Therefore, the objective of the present work was to compare Resting Energy
50 Expenditure (REE) under basal diet (BD) and challenging diet (CD) in which fat on sugar content
51 ratio was doubled. Diet-induced thermogenesis (DIT) and body compositions were also compared.
52 Energetic vs regulatory aspects of increasing fat contribution to total nutritional energy input were
53 essentially addressed through measures primarily aiming at modifying total fat amounts and not
54 the types of fats in designed diets.

55 *Methods:* In this randomized cross-over study, BD contained 10% proteins/30% lipids/60%
56 carbohydrates (fat on sugar ratio = 0.5) and was the imposed diet at baseline. CD contained 10%
57 proteins/45% lipids/45% carbohydrates (fat on sugar ratio = 1). Main and second evaluation
58 criteria measured by indirect calorimetry (QUARK RMR[®], Cosmed, Pavona; Italy) were REE and DIT,
59 respectively. Thirty four MOD patients were included; 22 (mean age 13.2±4.7 years, 50% female;
60 BMI 16.9±4.2 kg/m²) were evaluated for REE, and 12 (mean age 13.8±4.8 years, 60% female; BMI
61 17.4±4.6 kg/m²) also for DIT. OXPHOS complex deficiency repartition in 22 analysed patients was
62 55% for complex I, 9% for complex III, 27% for complex IV and 9% for other proteins.

63 *Results:* Neither carry-over nor period effects were detected ($p=0.878$; ANOVA for repeated
64 measures). REE was similar between BD vs CD (1148.8±301.7 vs 1156.1±278.8 kcal/day ; $p=0.942$)
65 as well as DIT (peak DIT 260 vs 265 kcal/day ; $p=0.842$) and body composition (21.9±13.0 vs
66 21.6±13.3 % of fat mass ; $p=0.810$).

67 *Conclusion:* Doubling diet fat on sugar ratio does not appear to improve, per se, energetic status
68 and body composition of patients with MODs.

70 **Keywords:** Mitochondrial disorders or diseases, Energy expenditure, Diet induced thermogenesis,
71 Body composition.

72

73 **1. Introduction**

74

75 Mitochondrial OXPHOS disorders (MODs) encompass a group of rare genetic disorders
76 affecting respiratory chain oxidative phosphorylation (OXPHOS) and resulting from mitochondrial
77 or nuclear DNA defects [1,2]. The OXPHOS system plays a key role in transferring energy from
78 macronutrients to ATP through a sequence of coordinated reactions by which macronutrients
79 are oxidized. OXPHOS comprises five major protein-lipid enzyme complexes which are located in
80 mitochondrial inner membrane and work locally in synergy with the labile electron acceptors
81 ubiquinone (an amphiphilic isoprenoid compound) and cytochrome *c* (a 12 kDa peptide). Electrons
82 generated during glucose, fatty acids and amino acid oxidations are channeled across complexes I
83 and II to be transferred sequentially to coenzyme Q10, complex III, and complex IV. Complexes I,
84 III, and IV use part of the energy produced by the electron transfer flux to extrude protons across
85 the mitochondrial inner membrane, from mitochondrial matrix to mitochondrial intermembrane
86 space. The resulting proton gradient is consumed with energy utilization and production by
87 complex V which condenses adenosine diphosphate and inorganic phosphate into adenosine
88 trisphosphate (ATP) [3,4]. These overall mechanisms and players for ATP production are impaired
89 in disorders affecting OXPHOS, jeopardizing various cell functions notably those which require a
90 high energy supply. [5]. Currently, no satisfactory curative treatment for MODs exists. Only
91 symptomatic treatments of MODs are available [2,7,8]. Interestingly, dietary modulations have
92 been considered as regards to the key-role of mitochondria in energy production from
93 macronutrients [9,10].

94 Increasing fat vs carbohydrate content in the diet has been recommended for the
95 treatment of MOD. Several reasons justify this recommendation. (i) Mitochondrial oxidation of
96 NADH is thought to be diminished in MOD [5,11] patients especially those affected with
97 respiratory chain Complex I (CI) deficiencies, it might be by-passed by FADH₂, an alternative carrier
98 of reducing equivalents, the electrons of which can enter the respiratory chain distal to complex I.
99 (ii) Supply in FADH₂ to the mitochondria can be increased (relative to NADH) by increasing the
100 amount of triacylglycerols and fatty acids in the diet. On the basis of stoichiometry of
101 mitochondrial oxidations, fatty acids yield a ratio of FADH to NADH of 0.5, whereas glucose yields
102 a much lower ratio of 0.2. In addition, fatty acids in contrast to glucose are endowed with
103 uncoupling properties towards OXPHOS [12]. Though uncoupling OXPHOS lowers net ATP
104 production by mitochondria, it virtually accelerates mitochondrial respiration through increased

105 electron transfer rates, a feature which improves mitochondrial oxidations regulated by the
106 NADH/NAD⁺ and FADH₂/FAD ratios.

107 In the wake of this rationale, we hypothesized that increasing fat to glucose ratio within an
108 isoenergetic and isonitrogenous diet should improve energy substrate oxidation in MOD patients.
109 As a corollary, this dietary measure should actually increase resting energy expenditure (REE), an
110 assumption also based on *in vitro* data on human cell [13,14] and animal studies [15], and *in vivo*
111 data obtained in complex I deficiency mitochondrial myopathy patients [16–19]. The aim of the
112 present study was, therefore, to study REE under basal diet (referred throughout the manuscript
113 to as the basal diet (BD)) challenged by a diet in which fat on carbohydrate content ratio was
114 doubled (referred to as the challenging diet (CD)) as a potential treatment in children with a MOD.
115 In these children, diet induced thermogenesis (DIT) and body composition were also measured to
116 get a better account for whole body energetic balances under the two diets.

117

118 **2. Materials and methods**

119

120 *2.1. Trial design and participants*

121 This 3-year prospective study (2008-2011) included MOD patients from the reference
122 centers for Inherited Metabolic Diseases of Lille and Paris (Jeanne de Flandre Lille University
123 Hospital ; Robert Debré Paris University Hospital). Inclusion criteria were : (i) Child or adult
124 between 5 to 21 years with MOD [20], (ii) MOD subjects given a stable balanced diet having the
125 characteristics of basal diet (BD : 60 % carbohydrates, 30 % lipids, 10 % proteins) for at least one
126 month. Subjects were excluded from the study when they required hospitalization or presented
127 with any acute condition (such as infection) known to interfere with energy metabolism
128 assessment.

129 As shown in flow CONSORT diagram [21] on (Figure 1), from 36 MOD subjects included over
130 a period of four years, 22 accepted compliance to the diets and examinations, and were analysed
131 for principal evaluation criterion (*ie* REE in Group 2), and from these 22, only 12 accepted to
132 undergo the secondary evaluation criterion (*ie* DIT in Group 3).

133 This study was a randomized cross-over study (Figure 2). Method used to generate
134 sequence was a simple randomisation by one-to-one allocation ratio. Subject randomisation was
135 performed using sealed envelopes. REE were measured at four time points (*ie* Visit 1: baseline,
136 Visit 2: after one month of basal or challenging diet ; Visit 3: after wash-out period consisted of
137 one month of BD ; and Visit: 4 after one month of basal or challenging diet; see Figure 2). Neither

138 carry-over effect, nor period effect was detected ($p = 0.878$; ANOVA for repeated measures). All
139 assessments were performed in two Clinical Investigation Centers (CIC-1403-Inserm-CH&U of Lille;
140 CIC-9202-Inserm-AP-HP of Paris). Before each assessment visit, all subjects arrived by car to the
141 Clinical Investigation Center at 8:00 h, being fasted from 20:00 h on the previous day. Weight and
142 height were first measured, and then compliance with the imposed diet was evaluated.

143

144 2.2. Nutrition protocol management

145 Intervention of the trial consisted of a CD diet period of one month (*ie* 10 % proteins, 45 %
146 lipids, 45 % carbohydrates). CD and BD diets were isoenergetic and isonitrogenous diets.

147 Each patient was randomly assigned to the basal diet (BD) for the control period *vs* the
148 challenging diet (CD) for the intervention period. Each one-month period was thereafter inversed
149 according to a cross-over trial. BD consisted of 10 % proteins, 30 % lipids, 60 % carbohydrates. To
150 reach this BD, research dieticians used a specific booklet communicated to subjects and/or their
151 parents to lower fat intake. CD consisted of 45 % lipids, 45 % carbohydrates ; 10 % proteins.

152 DIT was performed after a calibrated breakfast corresponding to 20% of recommended
153 daily energy intake according to Martin *et al's* formula [22]. According to the cross-over design,
154 breakfast had a BD in control period and a CD in intervention period. Basal content breakfast
155 consisted of bread, skimmed milk and jam. Challenging breakfast consisted of bread, full-cream
156 milk and chocolate paste. After breakfast, meals were re-weighted and diet energy supplies were
157 analysed by the difference between served and returned meals [23].

158 The assessment of energy intake and diet composition was performed using dietary record
159 technique previously reported [24]. The technique used a specific chart report form including how
160 to record the foods consumed, using size instruments such as pictures of graduated bowls, cups,
161 dishes, number of spoons. Each file corresponding to one day was separated in four parts:
162 breakfast, lunch, dinner and snack. Lunch and dinner were detailed as starter, main course, and
163 desert [25]. Both oral and written instructions were given to each patient and/or parents on how
164 to keep accurate records using the size instruments, and the parents assisted the children in
165 recording, identifying and quantifying the foods consumed. Subjects detailed daily food intakes on
166 the specific chart report form during 7 d [26]. The same project related dietician reviewed each
167 specific chart report. Quantification of meals and drinks were checked by the same trained
168 dietician, the parents and the child together. The dietician was well trained to identify both miss
169 and over-reporting and coherency of food records. The portion sizes were estimated using a three
170 dimensional portion size instrument with French current meal food photographs corresponding to

171 an exact BILNUT software (version 6 : Paris, France). This method has been shown to give unbiased
172 records of energy intake in lean subjects up to 9 y old and showed that children aged 8-15 y were
173 able to estimate food quantity to within = 10 % of the amount really eaten, suggesting that
174 children could quantify their food intake with reasonable accuracy [27].

175

176 2.3. Indirect calorimetry assessment of energy expenditure

177 The subject rested recumbent on a hospital bed watching a videotape for 15 min. Same
178 quiet cartoons or videos (depending on age range of the subject) were used for all the subjects
179 [28]. Oxygen consumption (V_{O_2} in mL/min) and carbon dioxide production (V_{CO_2} in mL/min) were
180 determined by using the open-circuit indirect calorimeter validated QUARK® RMR (Cosmed,
181 Pavona di Albano, Italy). The validity and accuracy measurements were guaranteed by yearly
182 calibration of the flow settings by the manufacturer of the calorimeter and guaranty a high
183 accuracy of QUARK® RMR between study centres [29]. Calibration of zero, span and delay
184 alignment of the O_2 and CO_2 gas analyzers was performed daily before each test using a certified
185 calibration gas.

186 The QUARK® RMR was equipped with a canopy hood for spontaneously breathing subjects.
187 With this device, flow rate was directly measured with a digital turbine flowmeter. Accuracy of the
188 flowmeter was 2%. Ventilatory rate was regulated directly by the QUARK® RMR. During each test,
189 the readings were controlled and eventually compensated by means of periodic automatic room
190 air calibrations. Response times of O_2 and CO_2 sensors were less than 120 ms. The O_2 analyzer was
191 a paramagnetic sensor, which had a measuring range from 0 to 30% in the canopy mode. Accuracy
192 of the O_2 sensor was 0.02 %. The CO_2 analyzer was an infrared digital sensor with a measuring
193 range standing from 0 to 10%. Accuracy of the CO_2 sensor was 0.02%. The flow meter which
194 detects the ventilation rate of the canopy was a bidirectional turbine with an 18 mm diameter.
195 The ventilation range was from 0 to 80 L/min. Accuracy of the flowmeter was 2%. QUARK® RMR
196 software utilized the Weir equation to assess Resting Energy Expenditure (REE) [30].

197 DIT was measured at two occasions (Visit 2 and Visit 4) after REE measurement. DIT was
198 derived from postprandial energy expenditure (PEE) as described by Dabbech M *et al* [31]. PEE
199 assessment started immediately after a calibrated breakfast (see above for energetic ratio). DIT
200 was computed by subtracting REE from PEE (*ie* $DIT = PEE - REE$) according to Dabbech M *et al* [31].

201

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204 *2.4. Assessment of body composition by Dual X-ray Absorptiometry*

205 The DXA (Dual X-Ray Absorptiometry) instrument was a Lunar DPX-IQ “pencilbeam” (Lunar
206 Radiation Corporation, Madison, WI, USA). Whole-body scan time was 560–590 s, and the
207 radiation dose was less than 0.03 *mrem* (¼ 0.3 *mSv*). Total body image acquisition and analysis
208 were obtained by following strictly manufacturer’s specified instructions. The use of two photon
209 energies reduced errors due to irregular soft-body-mass and body contours. Two photon energies
210 discriminated two substances in a given system to a high accuracy. When more than two
211 substances, the discrimination depended on the number of additional substances, their
212 attenuation characteristics, and their relative contents [32]. FFM (fat-free mass), FM (fat mass) and
213 weight were calculated using the software provided by the manufacturer, and was adapted for the
214 subject age.

215

216 *2.5. Regulatory requirement*

217 The protocol was approved by French appropriate regulatory authorities (Direction
218 Générale de la Santé; DGS 2007-A0018-50) and ethical review committee (Comité de Protection
219 des Personnes Nord-Ouest IV ; CPP 07/32). Study protocol was also declared to clinical trial.gov
220 register (NCT02385565). Participation in the study was voluntary and written informed consent
221 was obtained from both parents and children. Written informed consent was obtained by the
222 subject when patient was aged over 18 years.

223

224 *2.6. Statistical analysis*

225 Required sample size was computed using previously published data from complex I
226 deficiency (CID) mitochondrial myopathy patients [16,17] with REE = 1120 ± 93 Kcal/min in basal
227 condition (Glucose infusion). REE obtained in hyperlipid MOD condition (Triacylglycerol infusion)
228 were 5 % higher at 1175 ± 93 Kcal/min. We hypothesised a 10% difference of REE between basal
229 and challenging diets. According to the cross-over study design of the study and 80% statistical
230 power, the sample size was defined at 20 patients to detect a significant difference between diets.

231 Statistical analyses were performed using SAS V.9 (Cary, USA). According to a Shapiro-
232 Wilk test, all variables had a normal distribution. In this context, parametric test χ^2 for ratio, and
233 to a cross-over trial design: period effect was tested by ANOVA analysis for repeated measures,
234 carry over effect was tested by Student *t* test. Difference between two diets was tested by Student
235 *t* test when number of subjects was above 15 [33,34]. When number of subjects was under 15, we

236 used non-parametric tests: *ie* Wilcoxon for paired analyses and Mann-Whitney for non-paired
237 analyses.

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239

240

241 **3. Results**

242

243 *3.1. Anthropometric, biochemical defects and main clinics of enrolled patients*

244 Anthropometric characteristics of patients from each of the 3 groups defined in the flow
245 CONSORT diagram illustrated at Figure 1 are presented on Table 1. No statistical differences were
246 observed between included and analysed subjects for REE, and DIT measurements. Table 2 further
247 summarizes main clinical and biochemical results of respiratory chain activities in skeletal muscle
248 and/or cultured skin fibroblasts. The most frequent defect was complex I deficiency at 55% (n=12).
249 Table 3 presents a minimum set for anthropometric data description of patients with complex I
250 deficiency and those with other defects. There was no difference between groups excepted for Z-
251 score BMI which was significantly lower in the group of patients with defects other than complex I
252 deficiency.

253

254 *3.2. Study of patients undergoing REE measurements*

255 *3.2.1. Food intakes*

256 Food intakes in fat, carbohydrates and proteins were determined during the cross-over
257 periods in the 22 patients undergoing REE measurements (Table 4).

258 *3.2.2. Metabolic REE measurements and anthropometrics*

259 As mentioned in the experimental section, REE were measured at four time points (see
260 Figure 2). No carry-over effect and no period effect was detected for the present study ($p = 0.878$;
261 ANOVA for repeated measures).

262 Table 5 presents full metabolic data at baseline and after basal vs challenging diet
263 conditions. Measured REE were close to the predictive REE. No significant differences were
264 observed between the basal and challenging periods for any parameters (*ie* VO_2 , VCO_2 , REE, RQ)
265 even when these parameters were adjusted by body composition parameters (*ie* weight, fat mass,
266 fat free mass, muscle weight). Patient body composition parameters did not also differ between
267 basal and challenging diet periods for whole analyzed population (table 6). There were no
268 differences between basal and challenging diet periods for measured REE and anthropometrics in
269 patients with complex I deficiency nor in the group of patients affected by others defects. (Tables
270 7 and 8)

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276 3.3. Study of patients undergoing DIT measurements

277 3.3.1. Food intakes

278 In a subset of 12 subjects, it was possible to measure DIT. Table 9 presents respective
279 carbohydrate, lipid and protein contents of basal and challenging breakfasts. According to these
280 data, designed diet conditions (*ie* basal vs challenging) were in practice respected

281 3.3.2. DIT measurements

282 Figure 3 gives time-courses of DIT over a postprandial period of 250 *min*. Maximum DIT
283 was obtained at 100 ± 27 *min* after basal breakfast and 122 ± 29 *min* after challenging breakfast (p
284 = 0.861). No significant differences in DIT optima, areas under the curve, time durations of
285 postprandial periods were observed between basal vs challenging diet conditions. Similarly, RQ
286 during DIT measurements were not significantly different between basal and challenging diet
287 conditions (Figure 4).

288

289

290

291 4. Discussion

292 Because of the well-established ability of fats to induce mitochondrial biogenesis, we
293 aimed to test efficacy of an increased fat on carbohydrate ratio of the diet to manage nutrition in
294 MODs in order to improve energy expenditure metabolism [15–18]. Our study was designed to
295 determine to what extent and how REE, and secondarily DIT and body composition, may be
296 impacted by doubling fat vs carbohydrate contribution to nutritional energy intake. The collected
297 data do not confirm our primary hypothesis of a higher REE in challenging diet conditions.
298 Therefore, they do not *a priori* corroborate previous benefits suggested for high-fat diet in MODs
299 in a specific subset of patients using a specific type of fat enrichment. Our study present several
300 strengths (*i*) the cross-over design that is perfectly adapted to the aims of our study and the rarity
301 and heterogeneity of MODs, without period and carry-over effect, (*ii*) an adequate sample size, (*iii*)
302 a very well controlled diet, (*iv*) a well characterized population and (*v*) the use of gold standard
303 methodology based on previously validated tools (*ie* indirect calorimetry, body composition

304 analysis). Duration of the cross-over periods of one month was considered to be sufficient for a
305 high stability of energy metabolism and changes in REE and DIT. The absence of differences of REE
306 between basal and challenging diets observed in our study might be explained either by the wide
307 genetic heterogeneity usually observed in subjects with a mitochondrial disease [35], and masking
308 the power/homogeneity effect or by no impact of the challenging diet on REE, DIT and body
309 composition. In this respect, majority of previous trials have been performed on complex I MOD
310 [15,16,36,37] and, until now, trials mixing patients with distinct complex deficiencies were lacking.
311 The present study overcomes this gap and allows comparison with previous studies, some extra
312 statistical analyses were performed on REE and body composition in the subset of complex I MOD
313 patients included in our study. These extra analyses did not detect any difference in REE and body
314 composition in this subset of patients (Tables 7 and 8). This result does not reject the
315 heterogeneity of our population as a cause mentioned above for a lack of benefit of challenging
316 diet in MODs as even in Complex I defect, there is a huge genetic variability [38]. Concerning
317 durations of the cross-over period, one month was an adapted period to observe any changes in
318 REE and DIT in view of one week as a known successful period to normalize complex I activity in
319 malnourished patients [37]. We did not observe any difference in DIT (*ie* energy expenditure and
320 RQ) as Flatt observed in healthy subject [39,40]. The last hypothesis of the lack of impact of CD on
321 energy metabolism despite a higher amount of energy intake at breakfast (+ 37 % see Table 9)
322 might result from the fact that the CD tested was at a 45% instead of a 50% dietary fat content,
323 though no effect of CD was also observed by De Meer K et al [41]. An important feature also is that
324 our CD was primarily aimed at doubling fat vs carbohydrate contribution to nutritional energy
325 intake and not to enrich fat qualitatively with a specific type of fats. Using a specific type of fat
326 Roef *et al* [16] found that triacylglycerol infusion improved some metabolic parameters (*ie* VCO₂,
327 respiratory quotient) during physical exercise in a sample of patients with deficient complex I. The
328 significant effect observed in their study may be accounted for by several factors :

329 *(i)* a specific effect of triacylglycerol intravenous infusion which is more energetic
330 efficient than oral intake because of a high rate of systemic delivery and contents
331 in medium-chain triglycerides (MCT). MCT are more energetic efficient than long-
332 chain triglycerides because of their faster handling by metabolic pathways. This
333 advantage is used in ketogenic diets where high fat ratios force the body to use
334 fats instead of carbohydrates [42]. The underlying energetic change in oxidative

335 phosphorylation (OXPHOS) lies in fatty acids yielding a ratio of FADH to NADH of
336 0.5, whereas glucose yields a much lower ratio of 0.2

337 (ii) measurements performed during a physical exercise occur during sustained
338 substrate oxidation, allowing a better detection of differences in energetic
339 metabolism,

340 (iii) a specific population response (only 4 subjects in this study). The absence of
341 response in our population even if we analyzed a subgroup of complex I-deficient
342 patients might be attributed to the lack of mechanisms covered by parenteral
343 route, MCT and physical exercise which are all conditions absent from our
344 experimental protocols. This does not preclude that the ketogenic diet does not
345 improve mitochondrial oxidations as a source of metabolic energy, but this
346 feature unfortunately failed to be detected by our study. In this respect, the
347 lower fat enrichment of our CD vs classic ketogenic diet (43.6 % of fat in our CD
348 vs 90% in ketogenic diet) is another aspect to take into account.

349 As MODs may exhibit a wide range of symptoms including developmental delay, seizures,
350 vision and hearing impairments, autonomous nervous dysfunction, gastrointestinal signs,
351 endocrine disturbances and failure to thrive, a special interest of these symptoms were checked
352 during the study period. No adverse reactions, adverse events of special interest, or serious
353 adverse events were observed excepted some fatigue in 6 patients from 24 randomized patients
354 only during basal diet period (not significant). As a whole, doubling fat vs carbohydrate
355 contribution to nutritional energy intake does not improve, *per se*, energetic status and body
356 composition of patients with MODs but does not also impact negatively patients. However,
357 undernutrition observed in defects other than complex I could suppose a higher defect in weight
358 energy balance in these patients vs complex I-deficient patients.

359

360

361 5. Conclusion

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363 As a whole, this study does not provide evidence that doubling fat vs carbohydrate ratio of
364 the diet increases whole-body energy expenditure in patients with MOD. We were not able to

365 demonstrate a lowering of the metabolic challenge induced by substrate oxidation as a suggested
366 potential benefit in CD for mitochondrial diseases [5]. Our study suggests that underlying
367 mechanisms for a potential beneficial effect for CD does not a priori involve modulations of REE,
368 nor DIT. The wide heterogeneity of the studied population and/or the amount/quality of lipids in
369 the diet might play a role as well in the generated data and subsequent conclusions

370

371

372 **Conflict of Interest Statement & Statement of Authorship.**

373 Frédéric Gottrand has received consulting fees from Numico Clinical Nutrition, lecture fees
374 from SMS and grant support from Danone Research. The remaining authors state no conflict of
375 interest.

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380 **Acknowledgments**

381 We thank parents and patients who have taken part in the study. Pascale HINCKER & Sarah
382 KAMMENEY for diet composition management. We thank Christelle GUIMBER study nurse from
383 CIC for paramedical/logistical assistance. We thank Brigitte PORAS, José CANOMORALES, Stéphane
384 DESCHILDER, Nathalie DUQUESNOY, Jeanne-Marie DESCAMPS-BEN, Valérie HELD, Isabelle
385 CHARDON, Laurence CRINON for DXA acquisition and Dr Georges LION for DXA validation and
386 interpretation. We thank Muriel BEUVRY and Anne GAUTREAU (CIC-PT-1403-CH&U-Inserm de
387 Lille, France) for typing this manuscript.

388

389 **Funding**

390 Lille University Hospital was the trial sponsor of this study. This study was founded by a
391 grant from French ministry of health (PHRC interregional N°2003/1901).

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Table 1

Anthropometric characteristics of total included subjects and those undergoing REE and DIT measurements. Results are expressed as mean values \pm SD.

	Group 1 <i>Total included subjects</i>	Group 2 <i>Analysed subjects for REE</i>	Group 3 <i>Analysed subjects for DIT</i>	p-value <i>(group 1 vs 2)</i>	p-value <i>(group 1 vs 3)</i>
n	36	22	12	NA	NA
Female (%)	48	50	60	0.837 [#]	0.916 [∅]
Age : Year	13.2 \pm 4.7	13.2 \pm 4.6	13.8 \pm 4.8	1.000 [§]	0.823 [§]
Weight: kg	36.3 \pm 15.8	36.8 \pm 15.9	40.2 \pm 18.4	0.928 [§]	0.765 [§]
Height: cm	143.8 \pm 22.1	143.4 \pm 20.2	148 \pm 21.3	0.918 [§]	0.811 [§]
BMI: Kg/m²	17.0 \pm 4.1	16.9 \pm 4.2	17.4 \pm 4.6	0.924 [§]	0.875 [§]
Z-score BMI	-0.96 \pm 1.6	-0.94 \pm 1.68	-0.75 \pm 1.59	0.878 [§]	0.749 [§]
Fat mass: %	23.1 \pm 12.9	22.22 \pm 12.3	22.3 \pm 11.5	0.888 [§]	0.902 [§]
[∑] FFM: Kg	28.6 \pm 11.7	23.3 \pm 11.5	32.9 \pm 12.2	0.817 [§]	0.396 [§]
[∑] Muscle: Kg	26.9 \pm 10.3	22.1 \pm 12.4	30.8 \pm 9.4	0.902 [§]	0.753 [§]

BMI = Body Mass Index

NA = Not Applicable

NS = Not Significant

FFM = Fat Free Mass measured by [∑]DEXA

[#] = χ^2 test : [∅] = χ^2 test with Yates correction

[§] = Student *t* test

Table 2

Repartition of respiratory chain complex deficiencies in the group of REE analysed subjects along with their main clinical signs (n=22; group 2). Mitochondrial respiratory chain enzyme activities were assessed by biochemical measurements on skeletal muscle biopsy or on cultured skin fibroblasts; diagnoses of MELAS and MNGIE were made on the basis of molecular studies.

Complex deficiency	n	Neurological signs	Muscular signs	Neuro-sensitive signs
I	12*	7	1	0
III	2**	2	2	1
IV	6	6	0	0
MELAS ¹	1	1	0	0
MNGIE ²	1	1	0	0
TOTAL	22	16	3	1

¹MELAS = Myoclonic Epilepsy Lactate Acidosis and Stroke like episodes with mutation 3243A>G (mitochondrial DNA).

²MNGIE = MyoNeuroGastro Intestinal Encephalopathy with mutation C130T-G128A.

* 3 of 12 patients are simultaneously deficient for complexes I, II and III.

** 2 patients are simultaneously deficient for complexes III and IV.

Table 3

Comparison between anthropometric characteristics of respiratory chain-deficient subjects with and without complex I deficiencies in the group analysed for REE and body composition. Results are expressed as mean values \pm SD.

	Patients with complex I deficiency	Patients with other defects	p-value
n	12	10	NA
Female (%)	61	56	0.395 \emptyset
Age : Year	12.5 \pm 4.5	14.3 \pm 4.9	0.693 \S
Weight : kg	39.9 \pm 15.9	33.5 \pm 15.4	0.376 \S
Height : cm	142.6 \pm 18.3	144.4 \pm 23.1	0.927 \S
Z-score BMI	-0.06 \pm 1.6	-2.0 \pm 1.11	0.010\S

BMI = Body Mass Index

NA = Not Applicable

FFM = Fat Free Mass measured by ²DEXA

\emptyset = χ^2 test with Yates correction

\S = U-Mann Whitney test

Table 4.

Food parameters during cross over periods for REE analysed subjects (n=22 group 2). Results are expressed as mean values \pm SD.

	Cross-over periods			p-value (BD vs CD)
	Baseline ^a	BD	CD	
Energy Intake: Kcal/d	1722.2 \pm 366.1	1729.6 \pm 401.6	1950.0 \pm 593.5	0.089 [§]
Carbohydrates: %	55.0 \pm 4.0	53.8 \pm 4.9	43.1 \pm 3.1	<0.001 [§]
Lipids: %	29.3 \pm 3.6	31.1 \pm 2.9	43.6 \pm 2.5	<0.001 [§]
Proteins: %	15.1 \pm 3.4	15.1 \pm 3.3	13.3 \pm 2.3	0.24 [§]

^a Baseline is during a basal diet period

^b significant

BD = Basal diet : CD = Challenging diet

§ = Paired student *t* test

Table 5.

Metabolic parameters at rest of analysed subjects (n= 22 group 2) at baseline, after basal diet or after challenging diet. Results are expressed as mean values \pm SD.

	Cross-over periods			p-value (BD vs CD)
	Baseline	BD	CD	
VO₂				
VO ₂ ml/min	210.5 \pm 42.5	205.6 \pm 54.2	204.7 \pm 43.8	0.904 [§]
VO ₂ ml/min/kg of body weight	4.5 \pm 2.1	4.8 \pm 2.0	4.8 \pm 2.1	0.899 [§]
VO ₂ ml/min/kg of FFM	5.4 \pm 2.3	6.1 \pm 2.3	6.9 \pm 4.2	0.775 [§]
VO ₂ ml/min/kg of muscle	5.8 \pm 2.2	6.5 \pm 2.3	6.5 \pm 2.3	0.939 [§]
VCO₂				
VCO ₂ ml/min	243.2 \pm 55.5	235 \pm 60.1	240.7 \pm 51.9	0.842 [§]
VCO ₂ ml/min/kg of body weight	5.3 \pm 2.3	4.5 \pm 1.9	4.4 \pm 2.2	0.810 [§]
VCO ₂ ml/min/kg of FFM	6.4 \pm 2.4	5.6 \pm 2.0	6.2 \pm 3.8	0.436 [§]
VCO ₂ ml/min/kg of muscle	6.9 \pm 2.3	6.0 \pm 2.1	5.9 \pm 2.4	0.332 [§]
REE				
REE Kcal/d	1229.6 \pm 303.8	1148.8 \pm 301.7	1156.1 \pm 278.8	0.942 [§]
² pREE Kcal/d	1192.4 \pm 232.4	1191.0 \pm 246.7	1200 \pm 245.2	0.934 [§]
REE Kcal/d/kg of body weight	38.1 \pm 13.7	34.7 \pm 11.8	35.7 \pm 15.1	0.720 [§]
REE Kcal/d/kg of FFM	46.4 \pm 13.7	43.3 \pm 12.3	50.2 \pm 28.9	0.624 [§]
REE Kcal/d/kg of muscle	49.1 \pm 13.0	49.9 \pm 12.4	48.1 \pm 16.2	0.834 [§]
RQ				
mRQ	0.86 \pm 0.10	0.87 \pm 0.08	0.85 \pm 0.10	0.834 [§]
pRQ	0.87 \pm 0.11	0.88 \pm 0.10	0.84 \pm 0.10	0.729 [§]

^a Baseline is during a basal diet period

^b significant

BD = Basal diet; CD = Challenging diet

REE = Resting Energy Expenditure

pREE = Predictive Resting Energy Expenditure

FFM = Fat Free Mass

mRQ = Measured Respiratory Quotient (measured VO₂/measured VCO₂)

pRQ = Predictive Respiratory Quotient (theoretically calculated from the nutritional contents of diets in each class of energetic substrates [carbohydrates, lipids and proteins])

§ = Paired student *t* test

§ = Paired student *t* test

²Predictive REE calculated by Harris and Benedict equation

Table 6.

Body composition parameters of subjects (n= 22) undergoing REE evaluations at baseline, after one month of basal diet and after one month of challenging diet. Results are expressed as mean values \pm SD.

	Cross-over periods			p-value (BD vs CD)
	Baseline	BD	CD	
Weight : Kg	38.8 \pm 15.9	36.6 \pm 13.7	38.5 \pm 18.0	0.698 [§]
Fat mass : %	22.1 \pm 12.3	21.9 \pm 13.0	21.6 \pm 13.3	0.810 [§]
FFM : Kg	29.3 \pm 11.5	29.1 \pm 12.2	26.8 \pm 11.0	0.716 [§]
Muscle : Kg	26.9 \pm 10.3	26.6 \pm 11.1	26.5 \pm 11.0	0.810 [§]

FFM = Fat Free Mass

§ = Paired student *t* test

^a Baseline is during a basal diet period

^b significant

BD = Basal diet : CD = Challenging diet

Table 7.

Minimum set of metabolic and anthropometrics data in complex I deficient subjects (n= 12) undergoing REE evaluations at baseline, after one month of basal diet and after one month of challenging diet. Results are expressed as mean values \pm SD.

	Cross-over periods			p-value (BD vs CD)
	Baseline	BD	CD	
REE <i>Kcal/d</i>	1229.6 \pm 258.2	1139.5 \pm 250.3	1158.2 \pm 255.5	0.695 [§]
RQ	0.75 \pm 0.12	0.88 \pm 0.14	0.82 \pm 0.08	0.366 [§]
Weight: <i>Kg</i>	39.9 \pm 15.9	40.2 \pm 16.2	40.6 \pm 16.1	0.948 [§]
Fat mass: %	27.9 \pm 14.5	25.1 \pm 15.9	26.1 \pm 16.5	0.790 [§]
FFM: <i>Kg</i>	29.8 \pm 8.9	28.9 \pm 9.8	28.3 \pm 9.8	0.657 [§]
Muscle: <i>Kg</i>	26.9 \pm 8.2	26.5 \pm 9.3	25.8 \pm 9.1	0.929 [§]

FFM = Fat Free Mass

§ = Wilcoxon test

^a Baseline is during a basal diet period

^b significant

BD = Basal diet; CD = Challenging diet

Table 8.

Minimum set of metabolic and anthropometrics data of subjects with a respiratory chain defect other than complex I (n= 10) and undergoing REE evaluations at baseline, after one month of basal diet and after one month of challenging diet. Results are expressed as mean values \pm SD.

	Cross-over periods			p-value (BD vs CD)
	Baseline	BD	CD	
REE <i>Kcal/d</i>	1195.1 \pm 361.2	1166.5 \pm 378.5	1157.0 \pm 316.3	0.875 §
RQ	0.80 \pm 0.15	0.81 \pm 0.16	0.89 \pm 0.12	0.555 §
Weight: <i>Kg</i>	33.5 \pm 15.4	34.5 \pm 15.5	34.5 \pm 15.4	0.948 §
Fat mass: %	15.8 \pm 4.6	17.5 \pm 6.4	16.6 \pm 6.1	0.109 §
FFM: <i>Kg</i>	28.7 \pm 14.3	29.2 \pm 15.5	25.2 \pm 12.6	0.248 §
Muscle: <i>Kg</i>	26.7 \pm 12.7	26.8 \pm 13.7	27.3 \pm 13.2	0.185 §

FFM = Fat Free Mass

§ = Wilcoxon test

^a Baseline is during a basal diet period

^b significant

BD = Basal diet: CD = Challenging diet

Table 9.

Food parameters during cross over periods for DIT assessment (n = 12 ; group 3). Results are expressed as mean values \pm SD.

	Cross-over periods		p-value (BD vs CD)
	BD	CD	
Energy Intake*: <i>Kcal</i>	161.0 \pm 12.3	219.9 \pm 13.0	0.809 [§]
Carbohydrates: %	61.1 \pm 6.6	45.5 \pm 12.2	0.403 [§]
Lipids: %	28.5 \pm 10.3	42.3 \pm 11.1	0.878 [§]
Proteins: %	10.4 \pm 6.4	12.2 \pm 5.9	0.911 [§]

*Energy intake at breakfast for DIT measurement

§ = Wilcoxon test

BD = Basal diet

CD = Challenging diet

Figure 1 : Flow CONSORT diagram of screening, inclusion, randomisation and analysis rate in the study.

Figure 2 : Randomized cross-over design of the study.

Figure 3 : Evolution of diet induced thermogenesis (only group 3 ; n=12)

Figure 4 : Evolution of RQ during diet induced thermogenesis assessment (only group 3 ; n=12)

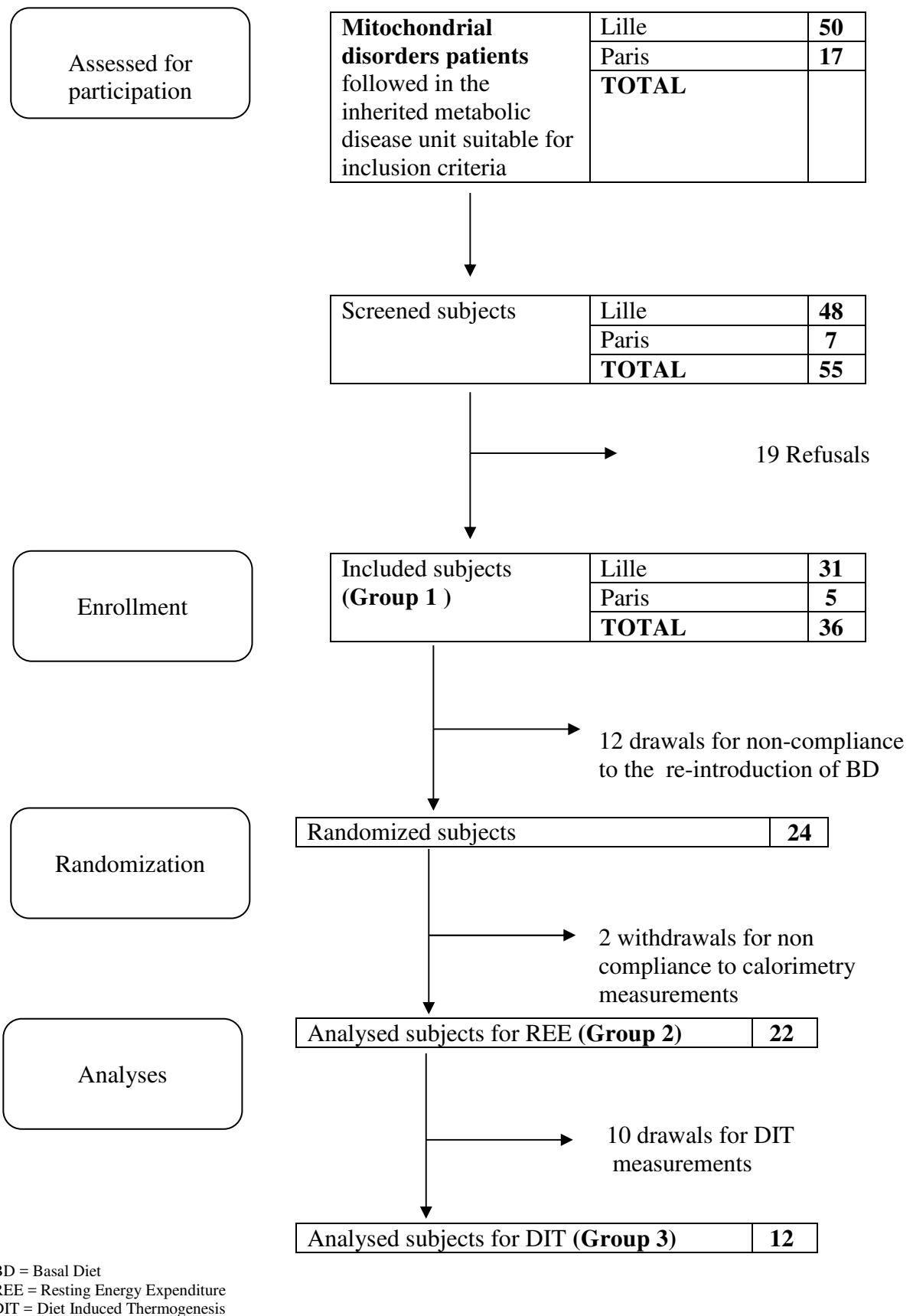
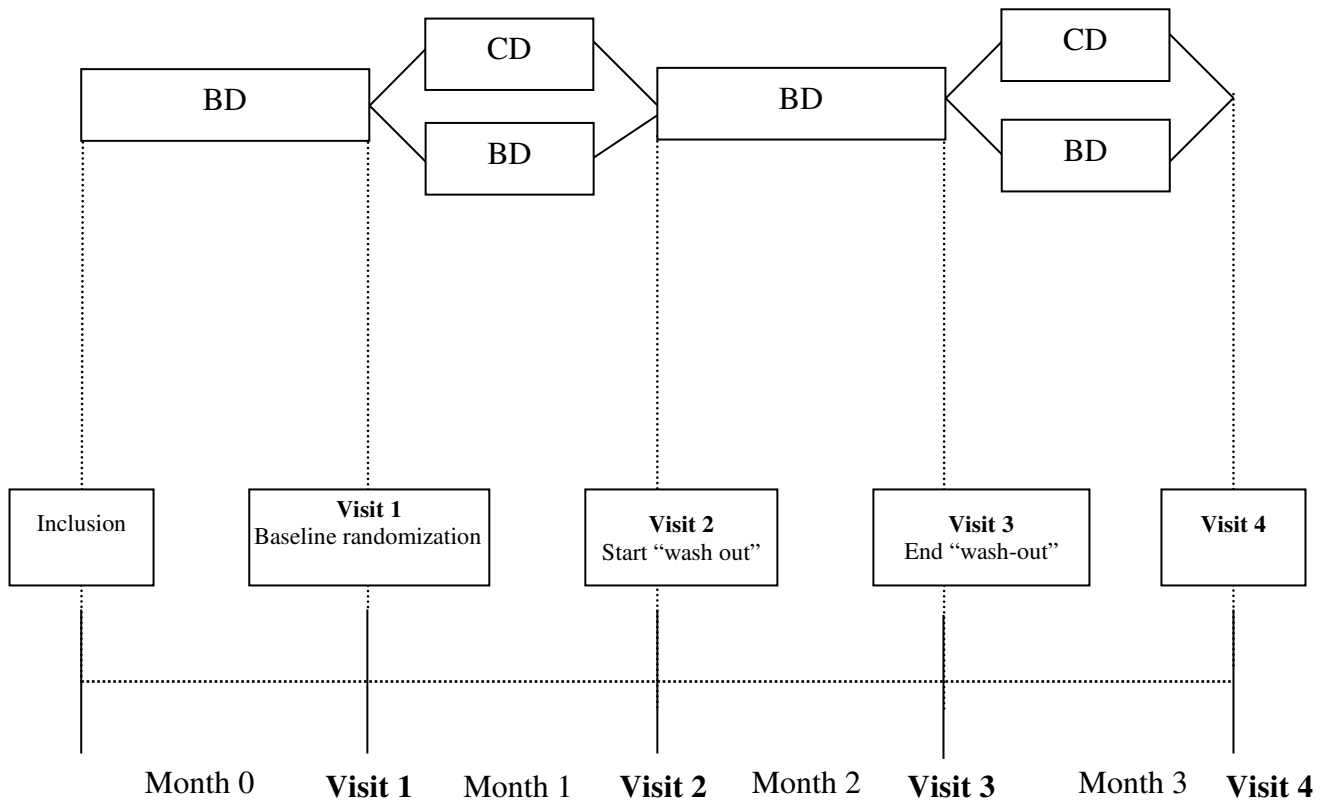


Figure 1 : Flow CONSORT diagram of screening, inclusion, randomisation and analysis rate in the study.



BD = Basal Diet
 CD = Challenging Diet

Figure 2 : Randomized cross-over design of the study.

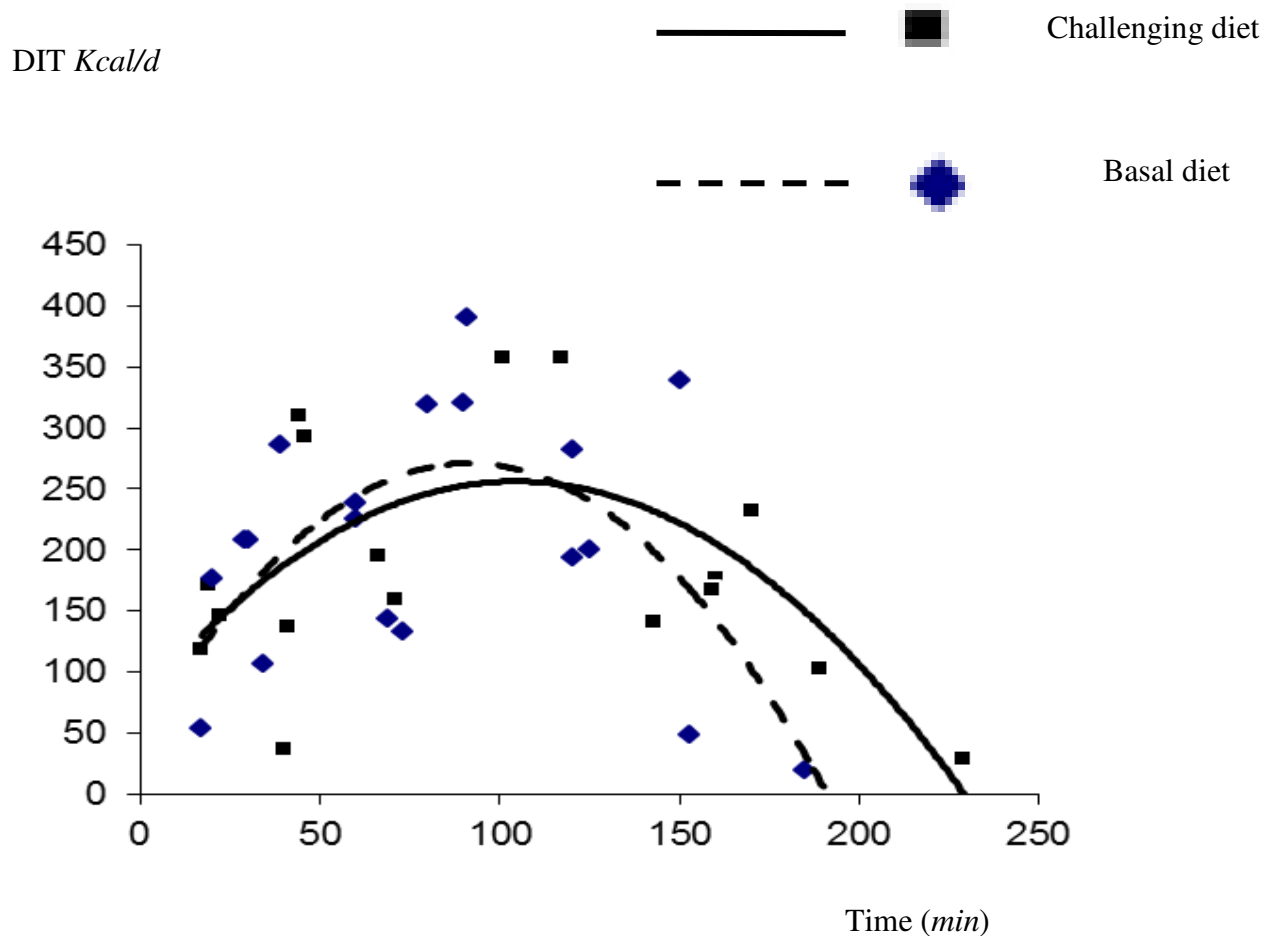


Figure 3 : Evolution of diet induced thermogenesis (only Group 3 ; n=12)

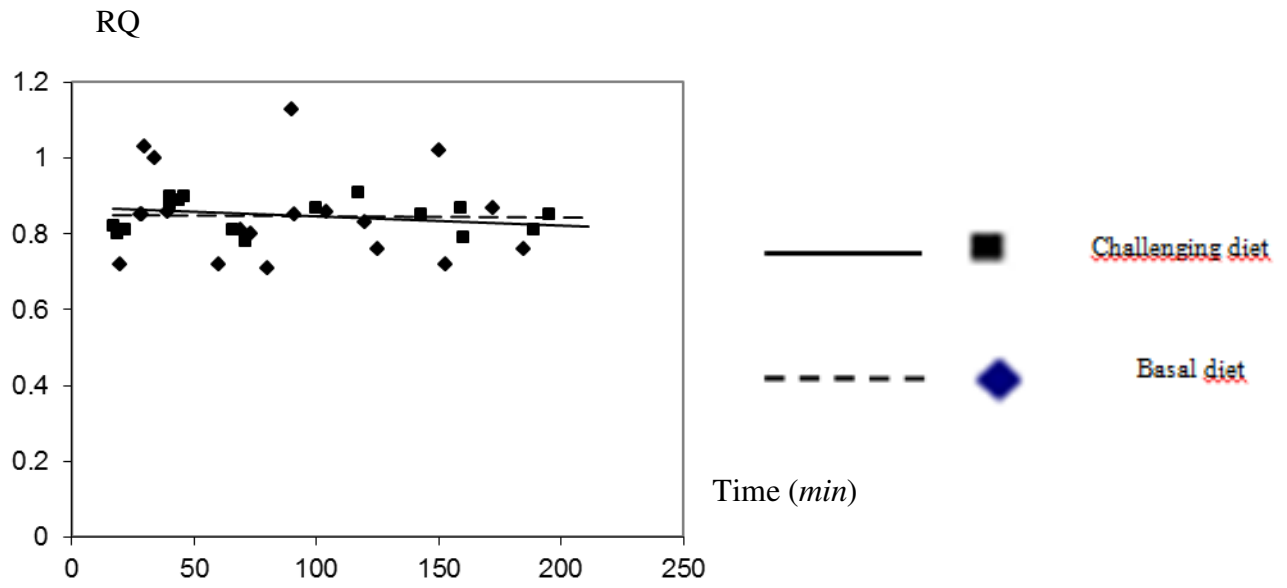


Figure 4 : Evolution of RQ during diet induced thermogenesis assessment (only group 3 ; n=12)