# Mammalian target of rapamycin inhibition impacts energy homeostasis and induces sex-specific body weight loss in humans

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## Abstract

**Background**  Previous data from a 2-year randomized controlled trial (CRAD001ADE12) indicated that mammalian target of rapamycin (mTOR) inhibition by everolimus slowed cyst growth in patients with autosomal-dominant polycystic kidney disease (ADPKD). During the trial, we noted body weight loss in some patients, particularly in women. We hypothesized that everolimus causes body weight reduction by reduced food intake and/or metabolic changes, which could lead to cachexia.

**Methods**  Within a sub-analysis of the CRAD001ADE12 trial, body weight course was investigated regarding sex-specific differences in 433 adult ADPKD patients (everolimus, $n = 215$; placebo, $n = 218$). One hundred four out of 111 patients who participated in the clinical trial centre in Berlin were evaluated under everolimus/placebo therapy (on drug: everolimus, $n = 48$; placebo, $n = 56$) and after therapy (off drug: everolimus, $n = 15$; placebo, $n = 18$). Eating habits and nutrient/caloric intake were evaluated by validated questionnaires. Systemic and local metabolism was evaluated in four patients after an oral glucose load (OGL) by using calorimetry and adipose/muscle tissue microdialysis.

**Results**  Within the 2-year CRAD001ADE12 trial, a significant body weight loss was observed in female patients on everolimus versus placebo ($P = 0.0029$). Data of the Berlin Cohort revealed that weight loss was greater in women on everolimus versus men ($P < 0.01$). After 9 months, women and men had lost $2.6 \pm 3.8$ and $0.8 \pm 1.5$ kg ($P < 0.05$) in body weight, respectively, and after 21 months, they had lost $4.1 \pm 6.6$ and $1.0 \pm 3.3$ kg ($P < 0.05$), respectively. On everolimus, caloric intake was significantly lower in women versus men ($1510 \pm 128$ vs. $2264 \pm 216$ kcal/day, $P < 0.05$), caused mainly by a lower fat and protein intake in women versus men. Cognitive restraints, disinhibition and hunger remained unchanged. In a subgroup of patients resting metabolic rate was unchanged whereas OGL-induced thermogenesis was reduced ($7 \pm 2$ vs. $11 \pm 2$ kcal, $P < 0.05$). Fasting and OGL-induced fat oxidation was increased ($P < 0.05$) on versus off everolimus. In adipose tissue, fasting lipolytic activity was increased, but lipolytic activity was inhibited similarly after the OGL on versus off everolimus, respectively. In skeletal muscle, postprandial glucose uptake and aerobic glycolysis was reduced in patients on everolimus.

**Conclusions**  mTOR inhibition by everolimus induces body weight reduction, specifically in female patients. This effect is possibly caused by a centrally mediated reduced food (fat and protein) intake and by centrally/peripherally mediated increased fat oxidation (systemic) and mobilization (adipose tissue). Glucose uptake and oxidation might be reduced in skeletal muscle. This could lead to cachexia and, possibly, muscle wasting. Therefore, our results have important implications for patients recieving immune-suppressive mTOR inhibition therapy.

**Keywords**  ADPKD; autosomal-dominant polycystic kidney disease; calorimetry; eating behaviour; everolimus; microdialysis

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# Introduction

Autosomal-dominant polycystic kidney disease (ADPKD) is the most common monogenic inherited kidney disease in humans. Depending on the population studied, 1:400 to 1:2500 life births are affected by this disease.[1–3] With deterioration of kidney function over time, it results in chronic kidney disease, which ultimately leads to end-stage renal failure in more than 50% of patients at the age of 50 years.[2] ADPKD is characterized by a progressive development of numerous fluid-filled renal cysts that originate from epithelial cells in renal tubuli. The disease is caused by mutations in the polycystic kidney disease (*PKD*)*1* and *PKD2* genes that encode the transmembrane proteins polycystin-1 and polycystin-2, respectively.[4] Mutations in *PKD1* and *PKD2* account for approximately 85% and 15% of ADPKD cases, respectively. After promising results in animal studies,[5] a large, randomized placebo-controlled trial was conducted in ADPKD patients without major co-morbidities. In this trial, the serine–threonine-protein kinase mammalian target of rapamycin (mTOR) was targeted by everolimus (CRAD001ADE12, EudraCT Number 2006-001485-16; ClinicalTrials.gov Number NCT00414440).[6] The trial showed that everolimus slowed cyst growth in ADPKD patients but failed to slow the progression of loss of kidney function (clearance). Numerous adverse events were observed in the everolimus-treated patients, for example, anaemia, leucopaenia and thrombocytopaenia, stomatitis and oral ulcers, and hyperlipidaemia.[6] As investigators of the Berlin trial site, we observed a dramatic reduction in body weight in several patients (mostly women) during the study. This sparked our interest on putative effects of everolimus in body weight reduction and changes of energy homeostasis. Previously, body weight loss has been observed in kidney transplant patients who were treated with the mTOR inhibitor rapamycin.[7] Moreover, the serine/threonine-kinase mTOR has been shown to play an important role in energy homeostasis by affecting central and peripheral signalling pathways, as has been shown in several animal studies.[8] It has been suggested that mTOR acts as an intracellular nutrient ('fuel') sensor in the hypothalamus for regulating food intake, hunger, satiation and satiety.[9–11] Chronic kidney disease has been shown to be associated with muscle wasting and cachexia.[12] End-stage renal disease is associated with a high prevalence of frailty and cachexia, increased morbidity and mortality.[13–15] Based on these data, we hypothesized that mTOR inhibition causes body weight reduction by reduced food intake and/or metabolic changes in response to the everolimus treatment. We were particularly interested in possible sex-specific differences within these effects. Therefore, during the CRAD001ADE12 trial, patients were asked to participate in an extended diagnostic approach with specific metabolic tests at two time points: (1) within the trial (*on drug*) and (2) after closing the trial (*off drug*) (*Figure 1*). Statistical analyses were performed after unblinding of the CRAD001ADE12 trial.

# Methods

## Body weight

Body weights of the trial participants were measured during the study visits and according to the CRAD001ADE12 trial protocol.[6] Body weights were measured at baseline and at 6, 12, 18 and 24 months *on drug* and at 36 and 48 months *off drug* (*Figure 1*). Body weights from these patients were provided by the primary investigator after completion of the trial.

## Food intake questionnaires

Berlin patients were asked to complete a food questionnaire to monitor changes in eating behaviour, regarding either hedonistic or homeostatic factors.

### Hedonistic factors
Cognitive and sensoric motivation of eating habits was evaluated by a Three-Factor Eating Questionnaire (TFEQ) and by food intake questionnaire (total energy and nutrient intake)[16] *on drug* and *off drug*. The TFEQ consists of 21 items regarding cognitive restraints of eating, 16 items regarding disinhibition and 14 items regarding hunger.

### Homeostatic factors (macronutrients and micronutrients)
The food questionnaire evaluated and analysed macronutrient and micronutrient intake by using the PRODI® 5.4 expert (Nutri-Science) and OptiDiet (Gesellschaft für optimierte Ernährung [GOE]) software to minimize errors due to underreporting.

## Metabolic monitoring

Metabolic monitoring was used to analyse systemic and tissue-specific changes in energy metabolism. After an overnight (12-h) fasting, an oral glucose load (OGL; 75 g in 300 mL of solution) was given to patients with a weight loss of more than 5% of body weight and suspected to be *on verum*. Systemic energy metabolism was measured by
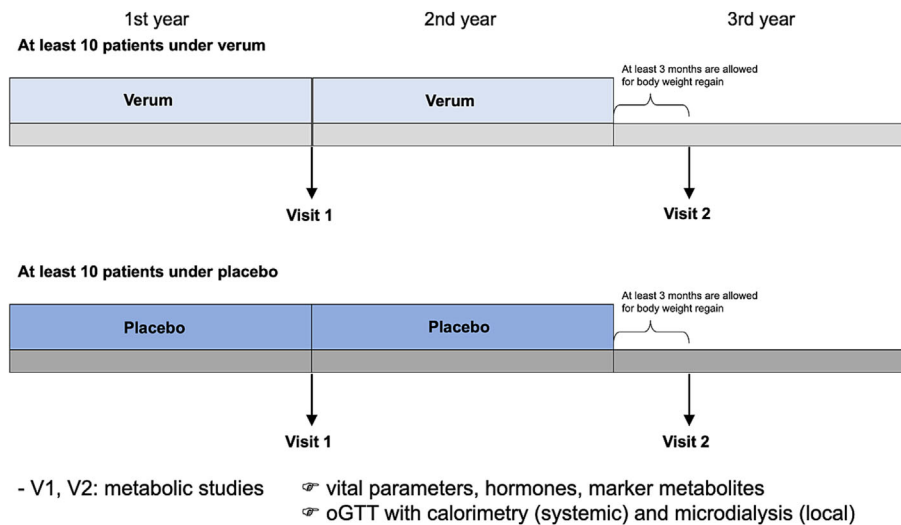
**Figure 1** Study design. oGTT, oral glucose tolerance test; V1 and V2, Visit 1 *on drug* and Visit 2 *off drug*; Verum, everolimus.

indirect calorimetry.[17] Tissue-specific metabolism was monitored by microdialysis. Additionally, plasma glucose and insulin levels were measured.

## Calorimetry

Oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$) were measured by using a ventilated canopy calorimeter (Deltatrac II, Datex Ohmeda, Helsinki, Finland) to assess changes in energy expenditure (EE), respiratory exchange ratio (RER; $VCO_2/VO_2$), and carbohydrate and lipid oxidation rates, respectively.[18] An RER of 1.00 indicates 100% glucose oxidation, and an RER of 0.70 indicates 100% fat oxidation. Throughout the study, subjects remained in a supine position. After a run-in period of 15 min, resting EE was measured for 30 min. After an OGL, measurements were continued for 120 min.

## Microdialysis

For monitoring tissue-specific metabolism, one microdialysis probe was inserted into abdominal subcutaneous adipose tissue at the level of the umbilicus and another one into the vastus lateralis muscle.[19–21] Perfusion of the tissues was started at a flow rate of 2 µL/min with Ringer's solution (Serumwerk Bernburg AG, Bernburg, Germany), containing 50 mmol/L of ethanol (EtOH; Braun Melsungen AG, Melsungen, Germany). Local tissue perfusion was monitored by using the ethanol dilution technique. After insertion of the probes, 60 min were allowed for tissue recovery from the insertional trauma and for baseline calibration of the perfusion system before commencement of the dialysis protocol. Dialysate marker metabolites such as glucose, lactate, pyruvate and glycerol were measured to assess changes in glucose supply, anaerobic and aerobic glycolysis, and lipolysis, respectively.[19–21] In situ dialysate recovery for dialysate me-

tabolites was ~30% in adipose tissue and 50% in skeletal muscle, as assessed by near-equilibrium dialysis. CMA/60 microdialysis probes and CMA/102 microdialysis pumps (CMA Microdialysis AB, Solna, Sweden) were used.

## Analyses and calculations

All blood samples were processed immediately in a refrigerated centrifuge at −4°C and aliquoted, and serum and plasma were stored at −80°C until analysis. Blood glucose and insulin concentrations, as well as routine parameters, were measured according to international standards. Perfusate (inflow) and dialysate (outflow) ethanol concentrations were measured with a standard spectrophotometric enzymatic assay. Dialysate concentrations of glucose, lactate, pyruvate and glycerol were measured with an automated analyser based on colorimetric principles (CMA/600, CMA Microdialysis AB). In situ dialysate recoveries for glucose, lactate, pyruvate and glycerol were ~30% in adipose tissue and 50% in muscle, as assessed by near-equilibrium dialysis.[22]

## Statistical analysis

Data are expressed as the mean ± standard deviation (SD) for anthropometric characteristics and as the mean ± standard error of the mean (SEM) for repeated measurements after the OGL. For statistical analysis, standard statistical software packages were used (StatView 5.0 [SAS Institute, Cary, NC, USA] and InStat, Version 4.0 [GraphPad Software Inc., San Diego, CA, USA]). $P$-values $< 0.05$ were considered to indicate statistical significance. The non-parametric Mann–

Whitney *U*-test for unpaired samples was used for comparing baseline characteristics of patients on verum and placebo, respectively. Wilcoxon signed-rank test was used to compare the response curves for plasma glucose and insulin and for EE, RER, and adipose tissue and skeletal muscle tissue dialysate concentrations of glucose, lactate, pyruvate and glycerol after the glucose load on patients *on drug* versus *off drug*.

# Results

## Patients

The CRAD001ADE12 trial cohort comprised a total of 429 ADPKD patients: 213 patients were *on verum* and 216 *on placebo*. Within this cohort, 104 men and 109 women were initially *on verum*, whereas 116 men and 100 women were initially *on placebo*. For more details, compare *Table S1*.

The CRAD001ADE12 trial sub-cohort from Charité – Universitätsmedizin Berlin comprised a total of 111 ADPKD patients (25.6% of the total CRAD001ADE12 trial cohort). Of these, 51 patients were *on verum*, and 60 patients were *on placebo*. Of these, 36 patients completed the food questionnaires: 18 patients *on verum* (9 men and 9 women) and 18 patients *on placebo* (11 men and 7 women; *Table 1*). Four patients (three women and one man) participated in the metabolic studies (calorimetry and microdialysis; *Table 1*). These four patients had a body weight loss between 7% and 32% on drug (suspected to be *on verum* because of potential side effects typically associated with everolimus). A secondary cause of weight loss, such as a malignant disease or chronic inflammation, was excluded in these patients clinically and by chest radiographs, abdominal ultrasound and laboratory testing. Unblinding revealed that these four patients were *on verum*. Extended diagnostic approaches (food questionnaires and metabolic studies) were carried out *on drug* and *off drug*.

**Table 1** Patients participating in the metabolic studies at the Berlin study centres

|  | Verum | | Placebo | |
| --- | --- | --- | --- | --- |
|  | Men | Women | Men | Women |
| Body weight |  |  |  |  |
| *On drug* | 26 | 22 | 27 | 29 |
| *Off drug* | 7 | 8 | 9 | 9 |
| Energy balance | 9 | 9 | 11 | 7 |
| TFEQ | 5 | 5 | 6 | 4 |
| oGTT | 1 | 3 |  |  |

Abbreviations: oGTT, oral glucose tolerance test; TFEQ, Three-Factor Eating Questionnaire.

## Body weight

### CRAD001ADE12 trial cohort (total)
Previously, weight loss has been reported in 14% of patients in the everolimus-treated group but only 3% in the placebo group ($P = 0.006$, calculated with the use of Fisher's exact test; *Figure 2*).[6] In the everolimus-treated group, body weight loss was mainly observed in the first 6 months on treatment, stabilized during the treatment period and reversed after cessation of treatment after 24 months (*Table S1* and *Figure 2*). Placebo-treated patients did not experience body weight gain (*Table S1* and *Figure 2*). Interestingly, a sex-specific effect was observed. Female patients *on verum* experienced a loss of body weight during everolimus treatment and regained weight *off verum* (*Table S1* and *Figure 2*). However, this effect could not be observed in male patients (*Table S1* and *Figure 2*).

### CRAD001ADE12 trial sub-cohort (Charité)
The trial sub-cohort at the Charité was representative and showed a similar reduction of body weight by everolimus compared with placebo as the CRAD001ADE12 total trial cohort (*Figure 3*, above, left). However, there was also a sex-specific significant body weight reduction of ~5% in women but only 2% in men (not significant [n.s.]) *on drug* (analysis of variance [ANOVA], $P < 0.05$, women vs. men; *Figure 3*, below, left). After 9 months on verum, women had lost 2.6 ± 3.8 kg and men had lost 0.8 ± 1.5 kg ($P < 0.05$), and after 21 months, they had lost 4.1 ± 6.6 and 1.0 ± 3.3 kg ($P < 0.05$), respectively (*Figure 3*, top). One woman lost 27 kg and another one lost 15 kg of body weight after 21 months on verum. In contrast, no body weight reduction and no sex differences were observed on patients in the placebo-treated group (*Figure 3*). Men in the placebo group gained ~2–3% of body weight between Months 3 and 18 of the trial treatment period. However, this effect was not statistically significant.

## Food questionnaires

### Hedonistic factors
Fully completed TFEQs *on drug* and *off drug* were retrieved from 10 trial participants of the everolimus-treated group (5 women and 5 men) and 10 participants of the placebo-treated group (4 women and 6 men). There were no differences in cognitive restraints of eating, disinhibition and hunger between the everolimus and placebo groups.

### Homeostatic factors (macronutrients/micronutrients)
During everolimus treatment, there were no significant differences in intakes of energy, macronutrients (carbohydrates, fat and protein), sodium and water *on drug* versus *off drug*,
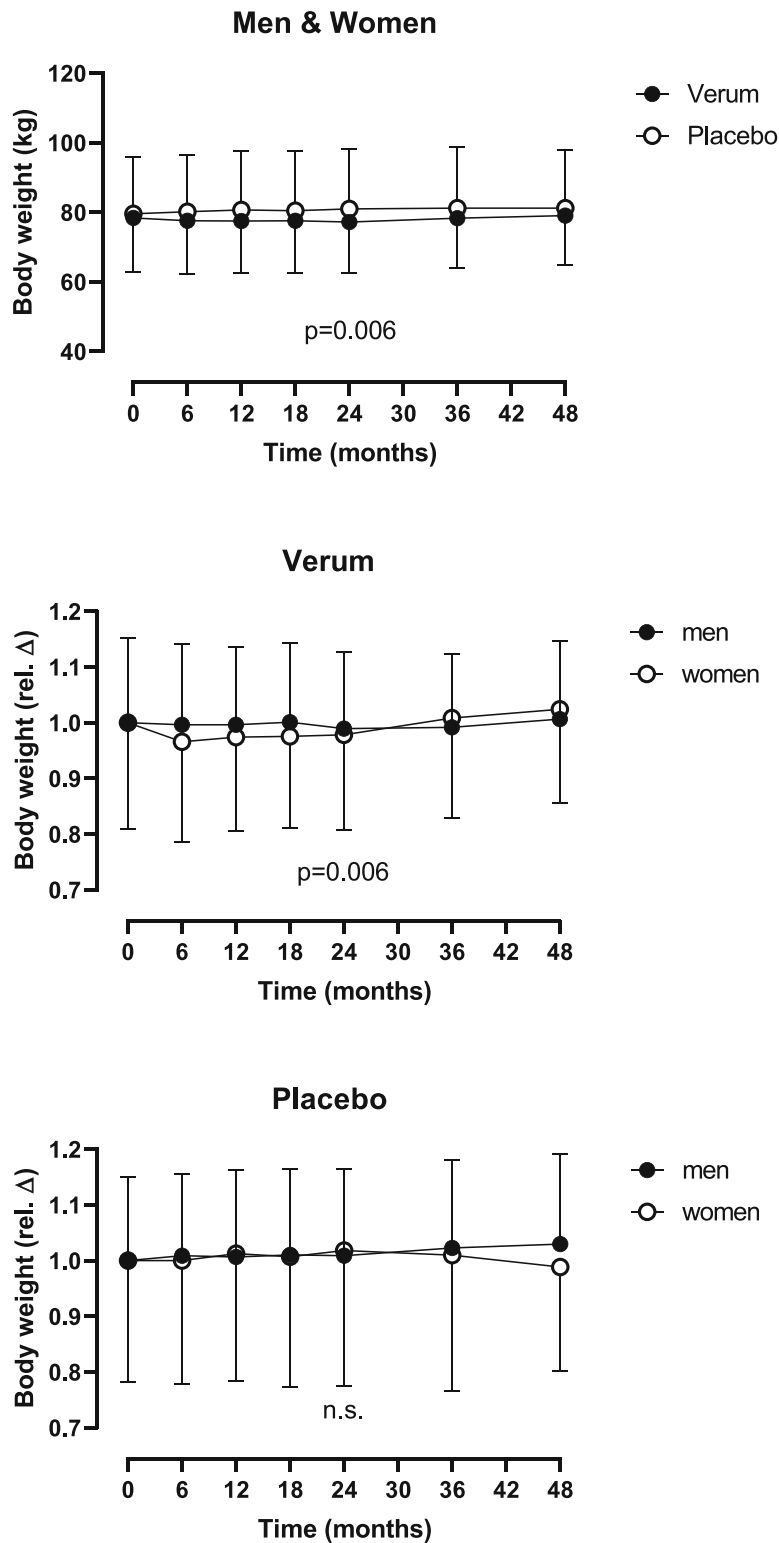
**Figure 2** Body weight development of patients under verum (everolimus) or placebo of the CRAD001ADE12 trial cohort over the total observation period of 48 months. Number of patients (total/men/women): at 0 months (baseline)—everolimus: 213/104/109, placebo: 216/116/100; at 24 months (end of treatment)—everolimus: As per our knowledge, thus far, there have been no observations of sex-specific body weight reduction during mammalian target of rapamycin inhibition with everolimus; 167/82/85, placebo: 194/110/84; at 48 months—everolimus: 130/60/70, placebo: 129/73/56. Weight loss has been previously reported in 14% of patients in the verum group but only 3% in the placebo group ($P = 0.006$, calculated with the use of Fisher's exact test). Data are given as mean ± SD. n.s., not significant. For further details, compare *Table S1*.
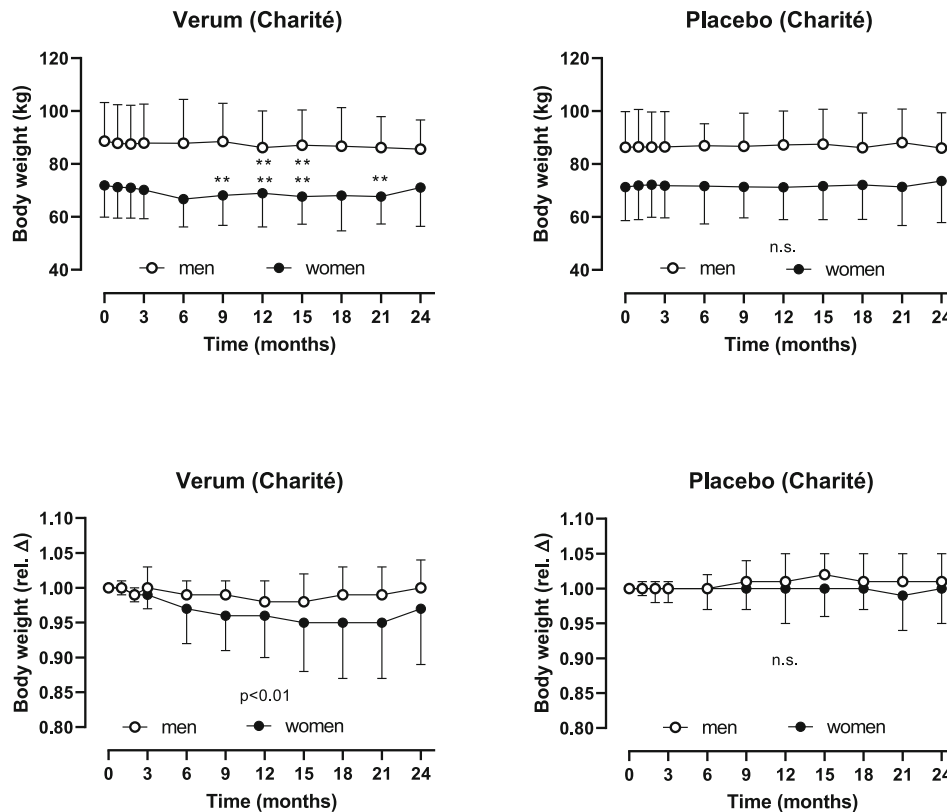
**Figure 3**  Body weight development of patients at Charité – Universitätsmedizin Berlin under verum (*n* = 60) or placebo (*n* = 51). There was sex-specific significant body weight reduction of ~5% in women but only 2% in men (top) *on drug* (ANOVA, $P < 0.01$, women vs. men). In contrast, no body weight reduction and no sex difference were observed on patients in the placebo group (bottom). Data are given as mean ± SD. **$P < 0.01$, 9, 12, 15 or 21 months *on drug* versus baseline (top).

**Table 2**  Daily intake of energy, carbohydrates, fat, protein, water and sodium of patients on verum or placebo during the on drug and off drug phases

|  | Verum | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|
|  | Men | | Women | | Men | | Women | |
|  | On drug | Off drug | On drug | Off drug | On drug | Off drug | On drug | Off drug |
| Energy (kcal/day) | 2264 ± 216[a] | 2031 ± 198 | 1510 ± 128[a] | 1755 ± 240 | 2229 ± 138 | 1883 ± 152 | 1865 ± 365 | 2152 ± 263 |
| Carbohydrate (g/day) | 272 ± 44 | 242 ± 30 | 179 ± 17 | 197 ± 31 | 227 ± 18 | 213 ± 18 | 211 ± 62 | 272 ± 53 |
| Fat (g/day) | 84 ± 6[a] | 77 ± 8 | 60 ± 9[a] | 70 ± 6 | 87 ± 6 | 68 ± 7 | 82 ± 6 | 76 ± 8 |
| Protein (g/day) | 92 ± 5[a] | 82 ± 8 | 55 ± 2[a] | 69 ± 14 | 88 ± 8 | 81 ± 6 | 64 ± 16 | 79 ± 3 |
| Water (L/day) | 2.54 ± 0.29 | 2.70 ± 0.26 | 2.42 ± 0.38 | 2.47 ± 0.43 | 3.00 ± 0.30 | 2.64 ± 0.24 | 2.23 ± 0.55 | 2.23 ± 0.46 |
| Sodium (g/day) | 3.36 ± 0.26 | 2.84 ± 0.42 | 1.97 ± 0.15 | 2.73 ± 0.96 | 3.12 ± 0.40 | 2.64 ± 0.11 | 3.99 ± 0.22 | 3.84 ± 0.13 |

*Note*: Verum group: five men and five women; placebo group: six men and four women. Data are given as mean ± SEM. Non-parametric Mann–Whitney *U*-test for unpaired samples.
[a]Values differ significantly ($P < 0.05$).

neither in men nor in women (*Table 2*). However, intakes of energy, fat and protein *on drug* were significantly lower in women versus men. There was also a tendency of lower intakes of energy, fat and protein in women *on drug* versus *off drug* (*Table 2*).

In placebo-treated patients, there were also no significant differences in intakes of energy, macronutrients, sodium and water *on drug* versus *off drug*, neither in men nor in women, and no significant differences between men and women *on drug* or *off drug* (*Table 2*).

## Metabolic monitoring

Systemic (calorimetry) and tissue-specific (microdialysis) metabolic responses were monitored during an OGL in four patients (three women and one man) on verum, both *on drug* and *off drug*.

### Blood parameter

Fasting glucose levels were 5.5 ± 0.6 mmol/L *on drug* and 3.7 ± 0.63 mmol/L *off drug* (n.s.). After the OGL, glucose levels

increased steeply and reached a maximum after ~60 min (*on drug*: 11 ± 1.8 mmol/L; *off drug*: 10.2 ± 1.5 mmol/L, n. s.). During the following 30 min, glucose levels remained at a plateau and then decreased slightly. At the end of the test, glucose levels in both groups were still above baseline, but not significantly different *on drug* (10.6 ± 2.1 mmol/L) versus *off drug* (9.4 ± 1.8 mmol/L; *Figure S1*). Baseline insulin concentrations *on drug* were 4.2 ± 0.5 μU/mL. After the OGL, insulin levels increased steeply and reached levels of 42.5 ± 13.6 μU/ mL after 45 min and 44.4 ± 14.4 μU/mL after 120 min (*Figure S1*). Unfortunately, *off drug* insulin values were not available because the samples were lost prior to analysis.

### Calorimetry

Resting EE was 4.18 ± 0.22 kJ/min *on drug* and 4.2 ± 0.32 kJ/ min *off drug*. After the OGL, EE increased continuously in both groups over 30 min. *On drug*, EE increased by 9% during 30 min after the OGL, started then to decrease and was still 8% above baseline after 120 min. *Off drug*, EE also showed a strong increase of 8% over 30 min, remained at this level and was still 11% above baseline after 120 min. After the OGL, relative changes in EE *on drug* were lower versus *off drug* ($P < 0.05$; *Figure 4*). Diet (OGL)-induced thermogenesis was 7 ± 2 and 11 ± 2 kcal/120 min *on drug* versus *off drug* ($P < 0.05$). Resting RER was 0.77 ± 0.03 *on drug* and 0.85 ± 0.03 *off drug* (*Figure 4*). After the OGL, RER decreased slightly during the first 30 min in both groups and then increased steadily to 0.87 ± 0.05 (*on drug*) and 0.96 ± 0.01 (*off drug*) after 60 min and further to 0.94 ± 0.06 (*on drug*) and 1.01 ± 0.06 (*off drug*) after 120 min. RERs were always significantly lower *on drug* versus *off drug* ($P < 0.05$). Therefore, fasting and postprandial fat oxidation rates were also always significantly higher *on drug* versus *off drug* ($P < 0.05$, data not shown).

### Microdialysis

In adipose tissue, baseline dialysate glucose was 1.26 ± 0.31 mmol/L *on drug* and 1.34 ± 0.32 mmol/L *off drug* (n.s.). After the OGL, glucose increased 2.5-fold above baseline levels *on drug* and *off drug* (n.s.; *Figure 5*). In muscle,

dialysate glucose tended to increase stronger *on drug* versus *off drug* after the glucose load (*Figure 5*).

In adipose tissue, baseline dialysate glycerol was 92 ± 16 μmol/L *on drug* and 99 ± 18 μmol/L *off drug* (n.s.). In muscle, baseline dialysate glycerol was 49 ± 8 μmol/L *on drug* and 54 ± 7 μmol/L *off drug* (n.s.). Glycerol decreased by ~50% after the OGL in both adipose tissue and muscle, without any significant differences *on drug* versus *off drug* (*Figure 5*).

In adipose tissue, baseline dialysate lactate was 0.36 ± 0.09 mmol/L *on drug* and 0.45 ± 0.10 mmol/L *off drug* (n.s.). After the OGL, dialysate lactate increased approximately three-fold above baseline *on drug* and *off drug* until the end of the test (*Figure 5*). In skeletal muscle, baseline dialysate lactate was 0.83 ± 0.08 mmol/L *on drug* and 0.93 ± 0.10 mmol/L *off drug* (n.s.). After the OGL, it increased in both groups ~1.5-fold and 2-fold above baseline levels *on drug* and *off drug*, respectively, until the end of the test (*Figure 5*). In muscle, dialysate pyruvate increased ~4.5-fold *off drug* and ~3-fold *on drug* (n.s.) after the OGL (*Figure 6*). Interestingly, baseline dialysate lactate/pyruvate ratio was higher *off drug* (~60) versus *on drug* (~40) but decreased on both treatments to ~20 during the test (*Figure 6*).

## Discussion

We observed that everolimus-induced inhibition of mTOR in ADPKD patients is associated with a significant body weight reduction. However, this effect was more pronounced in women versus men. Furthermore, in a subset of patients, the reduction of body weight in female patients is correlated with a reduced food intake, particularly a decreased fat and protein intake.

These effects were associated with reduced postprandial thermogenesis, together with increased resting and postprandial lipid oxidation during an OGL indicated by a reduced baseline and postprandial RER. The effects were associated with normal resting and postprandial adipose tissue and
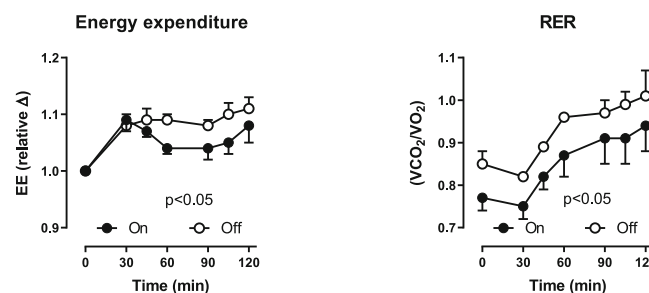


**Figure 4** Changes in energy expenditure (EE; relative) and respiratory exchange ratio (RER) after an oral glucose load of patients on verum (*n* = 4) in the *on drug* (on) and *off drug* (off) phases. Data are given as mean ± SEM. *P*-values refer to *on drug* versus *off drug*.
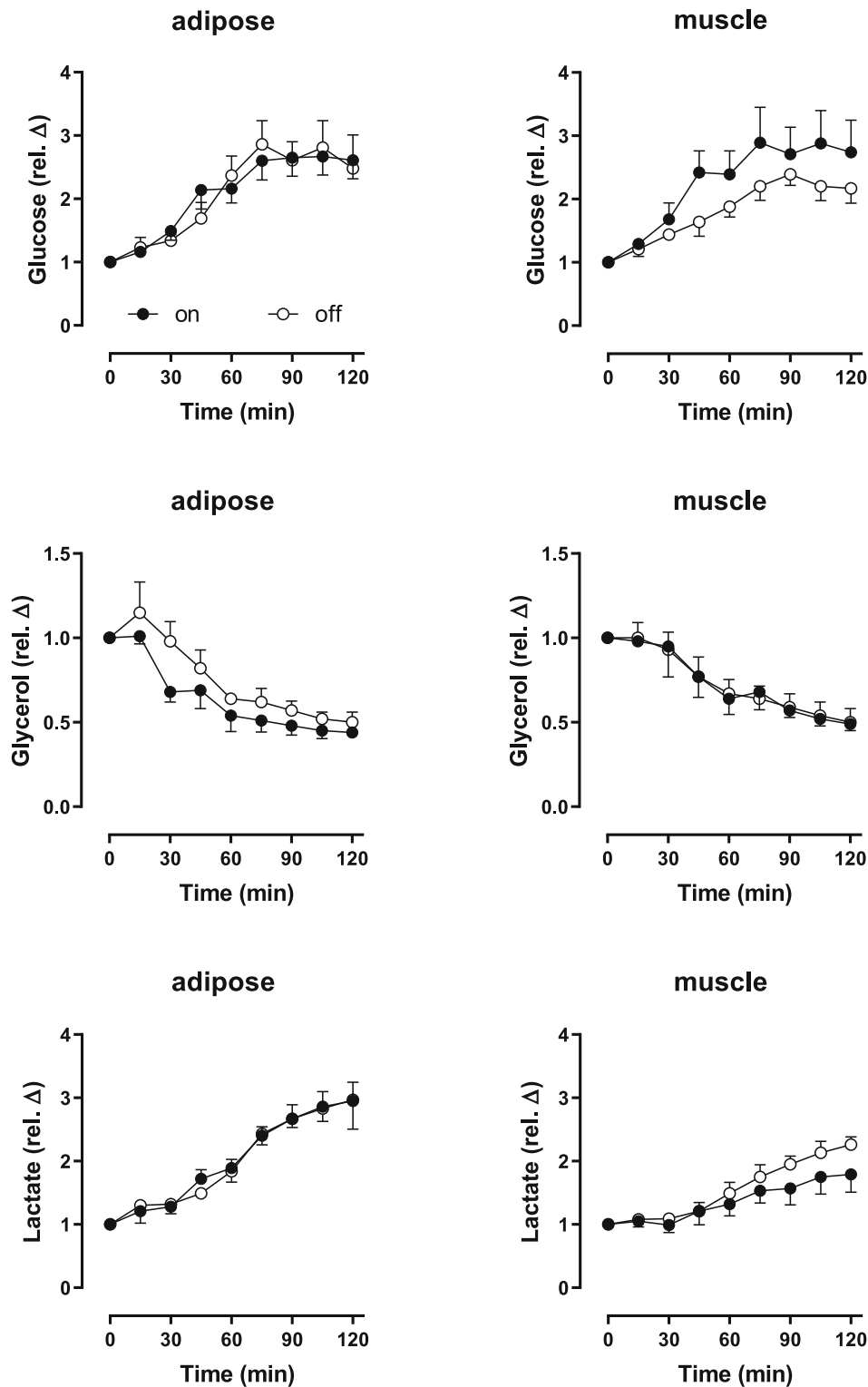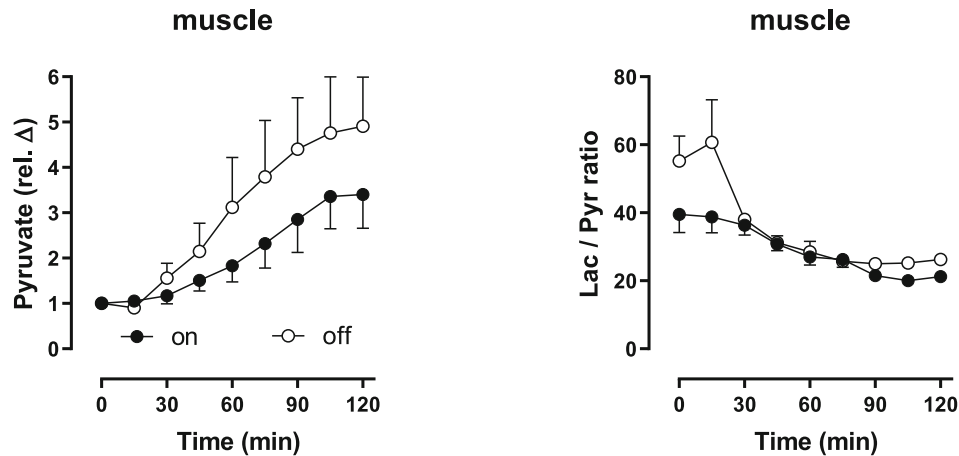
**Figure 5** Relative changes in dialysate concentrations of glucose, glycerol and lactate in abdominal subcutaneous adipose tissue and vastus lateralis muscle of patients on verum (*n* = 4) in the *on drug* (on) and *off drug* (off) phases. Data are given as mean ± SEM.

skeletal muscle metabolism. In muscle, postprandial dialysate glucose was slightly, but not significantly, higher on everolimus. No changes in insulin production and sensitivity were found. During glucose challenge, slightly elevated blood glucose concentration in conjunction with normal insulin levels was found *on drug*. Men just lost ~2% whereas women

**Figure 6** Relative changes in muscle dialysate pyruvate concentration and in dialysate lactate/pyruvate (Lac/Pyr) ratio of patients on verum (n = 4) in the *on drug* (on) and *off drug* (off) phases. Data are given as mean ± SEM.

lost ~5% of their initial body weight. This effect was mainly observed within the first 18 months of treatment. After the treatment period, an increase in body weight was observed in the verum group.

## Central mechanisms

As per our knowledge, thus far, there have been no observations of sex-specific body weight reduction during mTOR inhibition with everolimus. One explanation for the body weight reducing effect of everolimus, specifically in women, could be a centrally mediated modified eating behaviour. Female patients had, indeed, a reduced caloric intake of 350–400 kcal/day and, specifically, a reduced fat and protein intake under mTOR inhibition. Even considering that women on average have a lower energy intake than men,[23] this difference was not observed under placebo. Numerous amino acids—particularly leucin—are known to stimulate the mTOR signalling cascade.[9] While leucin stimulates food intake in the brain, it is conceivable that a reduced protein intake could elicit inhibitory mTOR effects, which in turn could indirectly further inhibit food intake. In mice, it has been shown that a very low protein diet caused decreased food intake despite activated hunger signalling and led to decreased body weight, partially due to inhibited hypothalamic mTOR signalling.[24] However, we could not further prove this hypothesis.

## Peripheral mechanisms

Body weight reduction is normally associated with a loss of both fat-free mass (25–30%) and fat mass (70–75%).[25,26] Unfortunately, we did not measure body composition. How-

ever, within a 5% body weight loss, up to 2% could be attributed to a loss of fat-free mass and, therefore, also to some loss of muscle mass. Myostatin and activin A induce muscle wasting by activating the ubiquitin proteasome system and inhibiting the Akt/mTOR pathway.[27] Both are found to show increased blood concentration in chronic kidney disease. Activin A, a member of the transforming growth factor-beta (TGF-β) protein family, decreases Akt/mTOR signalling and reduces muscle mass, leading to muscle wasting, sarcopenia and cachexia.[28] During the CRAD001ADE12 trial, baseline estimated glomerular filtration rate (eGFR) was reduced to 53.0 ± 19.8 and 56.0 ± 19.9 mL/min/1.73 $m^2$ in the verum and placebo groups, respectively. Unfortunately, we were not able to measure additional parameters like activin A in the trial participants. Interestingly, Solagna et al. used a tubular *Kif3a* deficiency mouse model in which the animals develop chronic kidney disease by growth of numerous renal cysts, weight loss and muscle wasting.[29] Polycystin-2 interacts with the microtubule-dependent motor kinesin-2 subunit *Kif3a*.[30] Polycystin-1 and *Kif3a* have been shown to participate critically in the response to myocardial infarction and fibrogenesis.[31] Interstitial fibrosis is also observed in Solagna's *Kif3a* mouse model expressing elevated levels of activin A. One could speculate that an activin A accumulation together with an additional pharmaceutical mTOR inhibition triggers metabolic changes, which could induce and/or aggravate weight loss, and, specifically, also a loss of muscle mass, which could finally lead to muscle wasting and cachexia. Further studies could shed more light on the role and interaction of activin A and mTOR inhibition.

We did not find any changes in resting metabolic rate. However, diet-induced thermogenesis was clearly reduced under everolimus. Additionally, resting and postprandial fat oxidation was significantly increased *on* versus *off* everoli-

mus, as indicated by the lower RER values *on drug*. Interestingly, baseline (fasting) dialysate glycerol levels were ~100 μmol/L *on drug* and *off drug*. These values were higher than usually observed in our studies (~60 μmol/L).[32] These increased fasting glycerol levels in these four patients indicate an increased lipolytic drive in adipose tissue. This could be in line with the increased fasting and postprandial fat oxidation.

### Sex-specific metabolic effects

Mechanisms of our observed sex-specific metabolic effects are unknown, but not surprising. In a model of sex-related postmenopausal cardioprotection, 17-β oestradiol was observed to modulate sirolimus effects on mTOR complex 2 (mTORC2) independently from the classical negative feedback loop of mTOR complex 1 (mTORC1) towards mTORC2 and phosphatidylinositol 3-kinase (PI3K) activity, which might be responsible for premenopausal cardioprotection.[33] Moreover, sirolimus causes downregulation of oestrogen receptor β in female mice and reduces mTORC2 by 60%.[34] These interactions could provide a clue to understanding sex differences in the mTOR regulatory system.

Although the underlying molecular mechanisms are unclear, we think our data have important clinical implications for patients treated with mTOR inhibitors. Our data indicate that reduced energy intake under everolimus may be more likely to be found with prior higher energy intake and/or the degree of overweight/obesity. With this respect, adipose tissue is known to produce leptin, which is functionally linked to the mTOR signalling. Women generally have higher leptin levels.[35] Luteinizing hormone (LH) can cause oestrogen production and thus stimulate mTOR.[36] Central regulation of food intake of mTOR is negatively regulated by mTOR,[8] which is opposite to the results of peripheral regulation, so that hyperphagia is inhibited by mTOR. In this case, hormones, such as LH, could intervene and enhance or attenuate mTOR effects.

### Limitations

Our study has several limitations. Our metabolic explorations were operated during an ongoing multicentre randomized clinical trial, and only a limited number of trial participants agreed to our metabolic tests. The observed weight loss in CRAD001ADE12 trial participants was unexpected and sparked our investigations. Unfortunately, we had no access to devices like bioimpedance analysis (BIA) or dual-energy X-ray absorptiometry (DXA) to assess changes in body composition. No tools for assessing hand grip strength were available to us. Further in-depth metabolic studies, including muscle and adipose tissue biopsies, and measuring biomarkers (leptin, 17-β oestradiol, fibroblast growth factor-21, LH and activin A), on a larger number of patients, would have required additional resources, which were not available. Also, a positive vote of the ethics committee would have been necessary. Therefore, only a few selected patients who lost a significant amount of body weight were subjected to our extended clinical and metabolic tests. The food questionnaire and food intake diary were self-reported by the patients and could be biased because of underreporting.

### Conclusions

In summary, mTOR inhibition by everolimus leads to sex-specific body weight loss, which is greater in women versus men. This is possibly caused by (1) centrally mediated decreases in energy, and fat and protein intake, and (2) by centrally/peripherally mediated increases in overall fasting and postprandial fat oxidation and increased fasting lipid mobilization. Both could explain the cachexia-like effect of everolimus. The body weight reduction could also be accompanied by a loss of fat-free mass (including muscle). Therefore, ADPKD patients, specifically women, on mTOR inhibitors could be at an increased risk for developing cachexia and, possibly, muscle wasting and sarcopenia. Our findings have important implications for patients receiving immune-suppressive mTOR inhibition therapy.

# Acknowledgements

# Conflict of interest statement

The authors declare no conflicts of interest.

# Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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