

Long-term hypophagic effect of chronic cannabis (Δ^9 -thc) administration on body weight progress investigated in a rat model

Abstract

Background: The use of cannabis to prevent weight gain or weight loss as unwanted side effects complementary to certain primary therapies inducing such side effects is still under study. Previous studies of different dose- and time-dependent D9-Tetrahydrocannabinol treatment approaches have shown contradictory effects on food intake behavior and body weight changes. Here we examined the effect of a daily dose of 3mg/kg over a period of up to 8.5month in a rat model which is equivalent to about 0.25grams over a period of 21.5years in terms of human life spans. Moreover, this effect was investigated in both, a native start generation and a D9-THC-biased offspring filial generation.

Methods: In this open, controlled study 48 nude rats (f+m) originating from two different generations were separated in D9-THC-treatment- and control groups. The daily administration of THC and control vehicle was done intraperitoneally. Body weight change was recorded at 14(SG) and 9(F1) time points throughout the study.

Results: In addition to a clear hypophagic effect found with long-term THC treatment, we observed an immediate cessation of this effect after settlement of the drug during recurrent lactation periods in both, SG and F1 generations. Thus the mechanisms involved in weight loss after chronic THC treatment at this dose-rate seem to remain reversible over a long period of time.

Conclusion: We conclude that long-term THC administration at a dose level of 3mg/kg might be suitable for complementary therapies effecting controlled weight loss when intended (e.g. treatment of type-2 diabetes, obesity, lack of sufficient sleep (insomnia), some types of cancer, etc.).

Keywords: d9-tetrahydrocannabinol, long-term treatment, hypophagia, complementary therapy, weight control

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Abbreviations: BW, body weight; F1, filial generation 1; SG, start generation; THC, d9-tetrahydrocannabinol

Introduction

In the course of a study examining the effects of chronic Δ^9 -THC administration on the gonadal vascularization and on the protective potential of the cornified envelope of the epidermis in nude rats (unpublished data) throughout different hereditary-biased filial generations we discovered an unexpected reversible hypophagic effect of THC caused by regular treatment intermissions during lactation and nursing periods of the native start generation (SG) and filial generation one (F1).

The potential of D9-Tetrahydrocannabinol (THC) to serve either to stimulate or retard appetite has still not been explored in full detail. Diverse findings of previous studies show contradictory effects of THC on body weight. Basically, dose-dependent and time-dependent differences have been reported. For therapeutic use it is of great interest as to how THC should be administered in order to obtain an optimal beneficial effect depending on the objective of treatment. Such objectives are on the one hand unwanted weight gain caused by appetite enhancement due to various first line therapies in the treatment of type-2 diabetes, lack of sufficient sleep (insomnia, stress)

etc. in turn resulting in reduced levels of the metabolism regulating hormones leptin and ghrelin as well as simple physical inactivity. On the other hand it is objected to treat patients suffering from severe appetite loss or muscle wasting (e.g. in cancer, cystic fibrosis, AIDS, anorexia etc.).

D9-Tetrahydrocannabinol (THC) is shown to have the ability, as partial agonist, to activate cannabinoid type-1 (CB1) and type-2 (CB2) receptors.¹⁻⁵ G-protein coupled cannabinoid CB1 receptors which are activated by the endocannabinoid neurotransmitters 2-arachidonoylglycerol and anandamide are located primarily in central and peripheral neurons, whereas CB2 receptors are predominantly in immune cells. CB1 receptors are also expressed by some non-neural cells in various tissues outside the brain (e.g. intestinal tract).⁶⁻¹¹ Thus, in addition to its psychoactive potential D9-THC also influences the ingestive behavior in animals and men. There is clinical evidence for the efficacy of inverse agonists of CB1 receptors (e.g. Rimonabant) for the improvement of the metabolic status in patients suffering from type-2 diabetes and dyslipidaemia but there are adverse psychiatric effects associated with this compound which caused its withdrawal from the market.¹²

D9-Tetrahydrocannabivarin (THCV), a naturally occurring analogue of THC, behaves as a neutral CB1 antagonist and was

found to produce hypophagic effects.¹³ The activation of CB2 receptor is suggested to improve glucose tolerance after a glucose load¹⁴ although other studies with CB2 knockout mice^{15,16} showed contrary results. Thus the exact role of CB2 receptors still remains unclarified. Bellocchio et al.¹⁷ reported a bimodal mode of action of endo- and exogenous cannabinoids in the control of stimulated food intake. According to the neural populations in which CB1 receptors are expressed, their pharmacology may vary. It is suggested that the orexigenic effect of a low dose of THC is mediated by CB1-dependent inhibition of glutamate release, whereas the hypophagic effect of higher doses of THC occurs via CB1-mediated inhibition of GABA release.

Williams et al.¹⁸ presented the first comprehensive dose-response and time-course analysis of exogenous cannabinoid effects on eating behavior in rats. With oral application of THC with doses in the range from 0.063 to 2mg/kg they obtained the most marked hyperphagic effect with a 1mg/kg dose. Although these authors found a dose-dependent hyperphagic effect, the study also clearly showed that this effect was an acute response to THC administration which occurred only within 1-2hours after treatment and which was abrogated after 4 hours. Similar dose responses to increased food intake have been presented by Glick & Milloy¹⁹ whereby in their study a dose of 2mg/kg decreased food intake. Appetite stimulation after single doses of THC has been found to be highly variable.²⁰ Some studies report a potential long-term benefit of THC on increased appetite in healthy individuals, cancer- and AIDS patients²¹⁻²³ even up to 42days. Mattes et al.²⁴ found a significantly elevated mean daily energy intake following chronic dosing (2.5mg/kg) by rectal suppository relative to acute dosing.

In the present study we focus on the long-term effect of medium dosage (3mg/kg) synthetic D9-THC (Dronabinol, 1mg/100 μ l) on body weight and its reversibility in a rat model. Contrary to most previous studies we note a hypophagic effect induced by chronic THC (i.p. daily) administration throughout a period of 284days. Comparing the life spans of rats and humans THC treatment of 8.5months in rats equals a human drug consumption period of about 21.5years.²⁵ Recurrent settlement of THC administration during nursing periods in female rats clearly shows an immediate reversal of this effect.

Material and method

Animals and study design

CR (Charles River) hairless mutation rat (CrI: CD-Prss8) of the start generation (SG) were subdivided into two groups:

- Δ^9 -Tetrahydrocannabinol (THC) treated adult nude rats (n=6 males and 6 females), and
- Healthy control adult nude rats (n=6 males and 6 females). The filial generation (F1) consisted of the same animal setting in each group. Animals within the THC groups (SG, F1) received 3mg/kg THC i.p. once a day for a period of 284days (SG), or 202days (F1). Dosage was readjusted to the actual individual body weight. Animals of the control group received the appropriate amount of sesame seed oil vehicle as a daily injection i.p. in order to maintain the same handling procedure. Dronabinol (synthetic D9-THC) was manufactured by dissolving cannabis resin in neutral oil at the pharmacy of the general hospital of Salzburg.

In periods of twomonths rats from the same group were kept pair wise and were allowed to mate. During the following lactation- and

nursing periods THC administration was interrupted. Rats were housed in an air-conditioned specific pathogen free environment with the temperature set at 20°C-24°C, 45-65% rH and a 12-hour-light: 12hour dark cycle (200lx). Due to the insufficient thermoregulation of nude rats high energy food (ssniff, Spezialdiäten GmbH, Germany) and water were provided ad libitum.

Assessment of body weight

Body weight (bw) was assessed using an electronic balance at 14(SG) or 9(F1) time points according to study events such as treatment onset, mating, nursing and scarification.

Statistics

Comparisons of body weight data between groups at the different time points were performed using two-tailed Student's t-tests with a power of 0.8 within 0.05 or 0.01 confidence intervals since all data were normally distributed.

Results and discussion

Generation SG

In the start generation (SG) significant differences ($p < 0.05$) in body weight (bw) between controls and THC treated animals were found in male rats only, first occurring after 1month of THC treatment. These differences in bw steadily increased until 8.5months of treatment when animals were sacrificed (Table 1) (Figure 1).

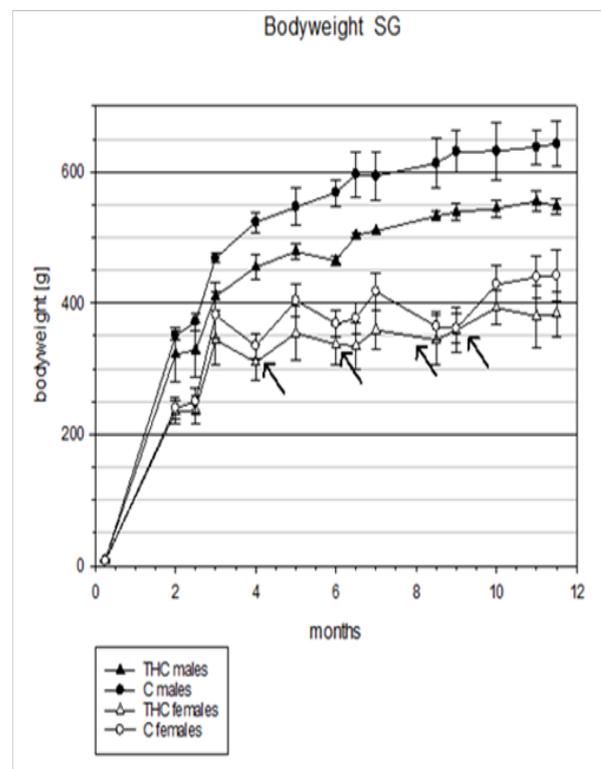


Figure 1 Start generation (SG): Comparison of body weight progress across 11.5months between chronic THC treated (3mg/kg bw i.p. daily injection) and control animals. Arrows indicate time points of female lactation periods (1month after giving birth without treatment) where differences of body weights between THC and control group were decreased. The difference in body weight progress of male animal groups (THC vs. C) increased steadily from the beginning until the end of the study.

No significant differences in bw occurred in female rats, probably due to four THC treatment interruptions (after 3, 5, 7, and 9 months) during late pregnancy and nursing. Even though there was no statistical significance in THC effects on reduced bw after 1 month of treatment comparable to those observed in male rats, a strong tendency was observed (Table 2) (Figure 1). Since the difference in bw after THC treatment interruptions with an average length of 31 days during lactation without THC administration decreased every time between treatment groups (Figure 1, arrows) and increased again

after reinstatement of THC treatment it may be suggested that the hypophagic effect of chronic THC administration on bw (reduction) is a reversible process.

Generation F I

Similar effects of chronic THC treatment resulting in highly significant lower body weights after 3 months of treatment ($p < 0.01$) in comparison to control animals were observed in filial generation 1-male animals (Table 3) (Figure 2).

Table 1 Body weight start generation: controls vs THC male rats

Treatment	Bw 0.25	Bw 2.0	Bw 2.5	Bw 3.0	Bw 4.0	Bw 5.0	Bw 6.0
	Months	Months	Months	Months	Months	Months	Months
C means	8g	350g	371g	469g	523g	468g	546g
C St Dev	1g	6g	13g	7g	16g	28g	20g
THC means	9g	322g	328g	410g	455g	464g	478g
THC St Dev	1g	41g	42g	21g	18g	11g	7g
Sign. Diff. p	0.288 n.s.	0.299 n.s.	0.166 n.s.	0.011*	0.008**	0.018*	0.001**
Treatment	Bw 6.5	Bw 7.0	Bw 8.5	Bw 9.0	Bw 10.0	Bw 11.0	Bw 11.5
	Months	Months	Months	Months	Months	Months	Months
C means	596g	594g	613g	630g	631g	638g	642g
C St Dev	34g	37g	37g	32g	43g	26g	35g
THC means	503g	510g	532g	538g	544g	555g	547g
THC St Dev	3g	0g	7g	13g	13g	16g	12g
Sign. Diff. p	0.009**	0.017*	0.021*	0.010**	0.029*	0.009**	0.011*

Bw SG, C vs THC male rats (t-test two-tailed, CI: $\alpha=0.05$, power: $\beta=0.8$, $n=6$ /group). THC treatment (3mg/kg bw i.p.) daily injection started at the age of 2 months. Data of body weight (bw) rounded; C, control group; THC, treatment group; n.s., not significant; *, significant ($p \leq 0.05$); **, highly significant ($p \leq 0.01$)

Table 2 Body weight start generation: controls vs THC male rats

Treatment	Bw 0.25	Bw 2.0	Bw 2.5	Bw 3.0	Bw 4.0	Bw 5.0	Bw 6.0
	Months						
C means	8g	240g	250g	382g	335g	404g	369g
C St Dev	1g	16g	20g	35g	19g	24g	21g
THC means	8g	235g	237g	344g	310g	354g	337g
THC St Dev	0.5g	17g	21g	37g	27g	42g	30g
Sign. Diff. p	0.576 n.s.	0.701 n.s.	0.441 n.s.	0.226 n.s.	0.203 n.s.	0.153 n.s.	0.206 n.s.
Treatment	Bw 6.5	Bw 7.0	Bw 8.5	Bw 9.0	Bw 10.0	Bw 11.0	Bw 11.5
	Months						
C means	377g	418g	365g	361g	428g	440g	442g
C St Dev	24g	28g	22g	22g	28g	32g	39g
THC means	335g	359g	344g	359g	393g	380g	383g
THC St Dev	35g	30g	38g	34g	26g	47g	34g
Sign. Diff. p	0.165 n.s.	0.071 n.s.	0.462 n.s.	0.936 n.s.	0.183 n.s.	0.147 n.s.	0.122 n.s.

Bw SG, C vs THC female rats: t-test two-tailed, CI: $\alpha=0.05$, power: $\beta=0.8$, $n=6$ /group; THC treatment (3mg/kg bw i.p.) daily injection started at the age of 2 months. Females were giving birth after 3, 5, 7, and 9 months (weight loss). Data of body weight (bw) rounded; C, control group; THC, treatment group; n.s., not significant; *, significant ($p \leq 0.05$); **, highly significant ($p \leq 0.01$)

Moreover, in female animals there were also significant ($p < 0.05$) differences in body weights which developed about 1 month after giving birth for the second time (i.e. months 6 and 6.5) (Table 4) (Figure 2). The difference in bw after THC treatment interruptions with an average length of 43 days during lactation without treatment

decreased every time between treatment groups (Figure 2, arrows) and increased again after reinstatement of THC treatment also in this generation. This again indicates that the hypophagic effect of chronic THC administration on bw (reduction) using a dosage of 3mg/kg is a reversible process.

Table 3 Body weight filial generation I: controls vs THC male rats

Treatment	Bw 1.0	Bw 1.5	Bw 2.0	Bw 3.5	Bw 4.0
	Months	Months	Months	Months	Months
C means	110g	217g	348g	474g	489g
C St Dev	1.5g	2g	3g	7g	14g
THC means	109g	207g	329g	425g	447g
THC St Dev	4g	6g	12g	9g	14g
Sign. Diff. p	0.768 n.s.	0.049*	0.054 n.s.	0.001**	0.022*
Treatment	Bw 5.0	Bw 6.0	Bw 6.5	Bw 7.5	
	Months	Months	Months	Months	
C means	527g	554g	565g	595g	
C St Dev	11g	7g	11g	16g	
THC means	465g	491g	502g	505g	
THC St Dev	10g	15g	16g	18g	
Sign. Diff. p	0.001**	0.002**	0.0001**	0.003**	

Bw F1, C vs THC male rats (t-test two-tailed, CI: $\alpha=0.05$, power: $b=0.8$, $n=6$ /group). THC treatment (3mg/kg bw i.p.) daily injection started at the age of 1 month. Data of body weight (bw) rounded; C, control group; THC, treatment group; n.s., not significant; *, significant ($p \leq 0.05$); **, highly significant ($p \leq 0.01$)

Table 4 Body weight filial generation I: controls vs THC female rats

Treatment	Bw 1.0	Bw 1.5	Bw 2.0	Bw 3.5	Bw 4.0
	Months	Months	Months	Months	Months
C means	88g	158g	252g	312g	331g
C St Dev	5g	2.5g	9g	16g	17g
THC means	89g	159g	283g	306g	318g
THC St Dev	1.5g	4g	67g	9g	7g
Sign. Diff. p	0.754 n.s.	0.909 n.s.	0.475 n.s.	0.554 n.s.	0.280 n.s.
Treatment	Bw 5.0	Bw 6.0	Bw 6.5	Bw 7.5	
	Months	Months	Months	Months	
C means	363g	365g	363g	376g	
C St Dev	12g	12g	8g	8g	
THC means	353g	328g	327g	358g	
THC St Dev	14g	12g	14g	18g	
Sign. Diff. p	0.395 n.s.	0.019*	0.017*	0.198 n.s.	

Bw F1, C vs THC Female rats (t-test two-tailed, CI: $\alpha=0.05$, power: $b=0.8$, $n=6$ /group). THC treatment (3mg/kg bw i.p.) daily injection started at the age of 1 month. Data of body weight (bw) rounded; C, control group; THC, treatment group; n.s., not significant; *, significant ($p \leq 0.05$); **, highly significant ($p \leq 0.01$)

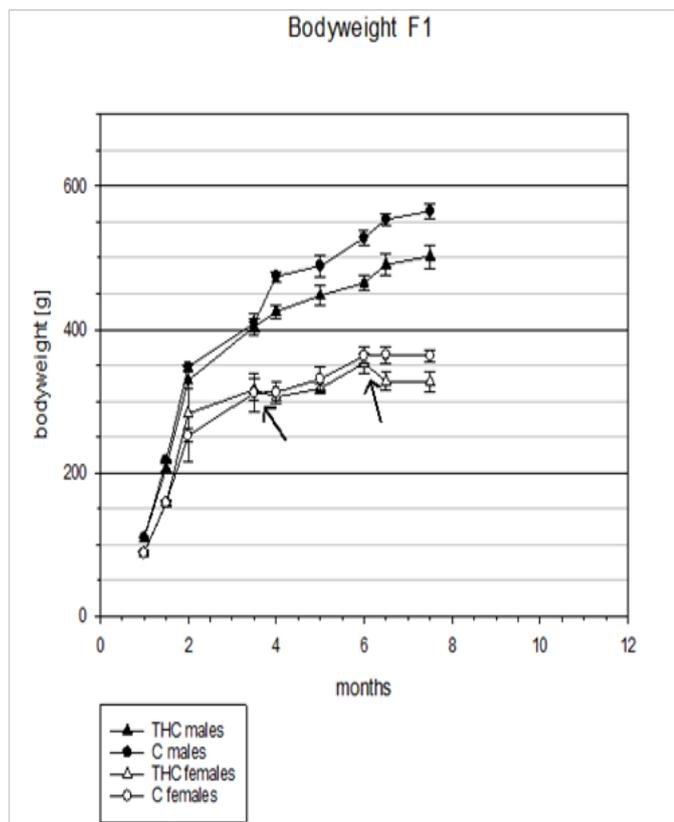


Figure 2 Filial generation I (F1), Comparison of body weight progress across 7.5months between chronic THC treated (3mg/kg bw i.p. daily injection) and control animals. Arrows indicate time points of female lactation periods (1month after giving birth without treatment) where no differences of body weights between THC and control group were found. After 3months of THC treatment the difference in body weight progress of male animal groups increased steadily until the end of the study.

Discussion

Since CB1 and CB2 receptors are coupled to G-proteins and the activation of these receptors by D9-THC induces a decoupling of G-proteins, in turn causing intracellular inhibition of the Adenylylcyclase enzyme and following second messenger cascades, this tremendously influences cell metabolism. Different dose- and time-dependent THC treatment approaches did show contradictory effects on food intake behavior and body weight changes. In our study we could clearly show that a dose of 3mg/kg over a long period resulted in markedly reduced body weight progress and thus might assist therapeutic strategies to decrease body weight in cases where intended. This observed hypophagic effect probably is due to either glucose tolerance- or drug absorption effects since there was no aversive food intake- or psychogenic behavior observed in the THC group. While short term administration of low doses (≤ 1 mg/kg) THC usually increases food intake,¹⁸ long term administration of a dose of 2mg/kg leads to weight loss¹⁹ which is in agreement with our findings. Recent publications of Le Foll et al.²⁶ and Sansone & Sansone²⁷ also proposed THC chronic treatment to produce weight loss, is associated with lower body mass index, and maybe a useful therapeutic for the treatment of obesity and its complications. Our results are in contrast to Abel²⁸ who concluded that after an initial period of weight loss, animals given cannabis begin to increase in body weight at a rate

similar to that of control animals. Other long-term studies^{21–24} have also found hyperphagic effects of chronic THC treatment which we cannot confirm here.

Moreover, we observed an immediate cessation of the long-term hypophagic effect after settlement of the drug during recurrent lactation periods in both SG and the F1 generation. Thus mechanisms involved in weight loss after chronic THC treatment at this dose-rate seem to remain reversible over a long period of time. Consumption of 3mg/kg per day (equals approx. 0.25 grams in humans) reflects dosages typical of light marijuana smokers whereas heavy smokers on average smoke a gram and a half to two grams of concentrate per day. The effect of such high doses on body weight change still remains unknown. In addition, different ways of drug administration (oral, rectal, smoke, intraperitoneal) may also cause divergent metabolic effects or food intake behavior and thus need to be explored in further detail.

We suggest that since no long lasting hypophagic effects of chronic THC treatment after settlement are observed, dose dependent chronic THC administration is suitable for complementary therapies effecting controlled weight loss when intended (e.g. complementary to various first line therapies in the treatment of type-2 diabetes, obesity, lack of sufficient sleep (insomnia), stress syndromes, some types of cancer, etc.).

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Conflict of interest

Author declares that there is no conflict of interest.

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