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Research Article

A Combination of Formoterol and the Histone Deacetylase Inhibitor AR42 has No Effects on Muscle Mass in Tumor-Bearing Rats

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ARTICLE INFO

Article history:

Received: 7 July, 2021

Accepted: 7 October, 2021

Published: 5 November, 2021

Keywords:

Cancer-associated cachexia

therapeutic approaches

muscle wasting formoterol

AR42

ABSTRACT

Background: Accelerated muscle and adipose tissue loss are two of the main aspects of cancer cachexia. β 2-agonists seem to be successful in the treatment of cachexia in experimental animals. The aim of the present investigation was to study the effects on body weight loss in tumor-bearing animals of a combination of formoterol and AR-42, an inhibitor of histone deacetylase (HDAC).

Methods: Rats were divided into two groups, namely controls (C) and tumor-bearing (T). TB group was further divided into four subgroups: untreated (saline as a vehicle), treated with Formoterol (F) (0.3 mg/kg body weight in saline, subcutaneous (s.c.), daily), treated with AR-42 (A) (20 mg/kg body weight in olive oil, intragastric (i.g.), only the last 4 days), and double-treated (TFA) with Formoterol (0.3 mg/kg body weight, subcutaneous (s.c.), daily) and AR-42 (20 mg/kg body weight in olive oil, intragastric (i.g.), only the last 4 days). 7 days after tumor transplantation, muscle weights, grip force and total physical activity were determined in all experimental groups.

Results: The presence of the Yoshida AH-130 ascites hepatoma induced severe muscle wasting in rats. Treatment of the tumor-bearing animals with the β 2-agonist formoterol (0.3 mg/kg), resulted in a significant improvement in the cachectic state of the animals. Treatment of the tumor-bearing animals with AR42 did not result in any effects on muscle wasting in the cachectic rats. Furthermore, the combination of formoterol and AR42 showed no additional effects to those observed with just formoterol.

Conclusion: The results presented question the previously described effects of AR42 on cancer cachexia, probably due to its effect on tumor growth.

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Background

A consensus group defined cachexia as a “complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity” [1]. From 50 to 80% of cancer patients experience the

cachexia syndrome. In fact, cachexia is a useful tool for survival prediction, being held responsible for more than 20% of the deaths of cancer patients [2]. It is directly responsible for a reduction in physical activity and quality of life and decreases the efficacy and outcome of anticancer therapy [3-5]. Both adipose tissue and muscle weights are reduced during cancer cachexia; however, muscle wasting is the main event. In fact, the loss of body weight and muscle mass are directly involved not only with survival but also the physical performance of the patient [6].

Many therapeutic approaches and strategies have been described to treat the cachexia syndrome, but, unfortunately, none of them are able to totally reverse the weight loss. Basically, the different targets addressed

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in the treatment of the syndrome are counteracting anorexia and/or neutralizing metabolic disturbances [7, 8]. Concerning the neutralization of the metabolic alterations, β 2-agonists, formoterol in particular, had important anti-cachectic effects [9]. The mode of action of this drug is based on its ability to prevent muscle wasting by inhibiting proteolysis and apoptosis in skeletal muscle. Thereby, formoterol decreased the activation of the ubiquitin-dependent proteolytic system, the main mechanism activated in muscle wasting conditions, as well as decreased muscle apoptosis in tumor-bearing animals [9, 10]. The anti-wasting effects of the drug were also observed in terms of total physical activity and grip force, thus resulting in an improvement in physical performance in cachectic tumor-bearing rats [11]. In humans, the combination of formoterol and the orexigenic drug megestrol acetate also resulted in a promising therapy in cancer cachexia [12].

Histone deacetylases (HDAC) regulate gene transcription through the elimination of acetyl groups presents in lysine, increasing the positive charge and consequently its affinity for DNA (which is negatively charged) [13]. Modification of histones by acetylation plays a key role in epigenetic regulation of gene expression and is controlled by the balance between histone deacetylases (HDAC) and histone acetyltransferases (HAT). HDAC inhibitors induce cancer cell cycle arrest, differentiation and cell death, reduce angiogenesis and modulate immune response [13]. In fact, AR-42, an inhibitor of HDAC, has been proved to have antitumoral effects in both hematologic and solid tumor malignancies, and this effect has been investigated in clinical trials for the treatment of patients with lymphoma, multiple myeloma and acute myelogenous leukemia [14-25].

Additionally, another function for HDAC has been described: it can modify the acetylation degree of non-histone proteins, such as transcription factors. This is the function that seems to be interesting for treating cancer cachexia. Inhibiting HDAC through AR42 administration could be a good therapeutic tool to stop muscle wasting, because acetylation of transcription factor FoxO, which is involved in the ubiquitin-dependent proteolytic system that includes genes such as MuRF-1 and atrogin-1, could stop muscle wasting programme [26]. A recent study shows how AR-42 treatment allows the recovery of basal expression of those genes and therefore, it stops the symptoms associated with cancer cachexia [27]. Other HDAC inhibitors (such as MS-275) prevent contractile dysfunction during skeletal muscle disuse and reduces the extent of fiber atrophy [28]. Another potential effect of HDAC inhibitors is that these compounds could behave as exercise mimicking agents (a well-known strategy against cachexia) [29]. Bearing all this in mind, the aim of the present investigation was to explore if the combination of formoterol and AR42 has any synergistic effect on cancer-related cachexia.

Materials and Methods

I Animals

6 weeks old male Wistar rats (Harlan, Barcelona, Spain) were housed in individual cages and maintained at a constant temperature of 22 ± 2 °C with a regular light-dark cycle (light from 08:00 a.m. to 08:00 p.m.) and free access to food and water. Experimental cachexia was obtained through i.p. injection of 100×10^6 AH-130 Yoshida ascites hepatoma

cells obtained from exponential tumors as described previously [30]. Food intake was measured daily. The experimental protocol was approved by the Ethical Committee of the University of Barcelona and all animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals [31].

II Experimental Design

Rats were divided in two groups, namely controls (C) and tumor-bearing (TB). T group was further divided into four subgroups: untreated (T) (saline/olive oil as a vehicle), treated with formoterol (F) (0.3 mg/kg body weight in saline, subcutaneous (s.c.), daily), treated with AR42 (A) (20 mg/kg body weight in olive oil, intragastric (i.g.), only the last 4 days), and double-treated (F+A) treated with formoterol (0.3 mg/kg body weight, subcutaneous (s.c.), daily) and AR42 (20 mg/kg body weight in olive oil, intragastric (i.g.), only the last 4 days). Seven days after tumor transplantation, animals were weighted and anaesthetized with an intraperitoneal (i.p.) injection of ketamine/xylazine mixture (3:1) (Imalgene® and Rompun® respectively). Tumor volume and total cell number were assessed at the day of sacrifice. Tissues were rapidly excised, weighted, and frozen in liquid nitrogen.

III Biochemicals

Formoterol was kindly provided by Industriale Chimica s.r.l. (Saronno, Italy), AR42 ((S)-(+)-N-hydroxy-4-(3-methyl-2-phenyl-butylamino) benzamide was obtained from Arno Therapeutics (Flemington, New Jersey) [32].

IV Grip Force Assessment

Skeletal muscular strength in rats was quantified by the grip-strength test [33]. The grip-strength device (Panlab-Harvard Apparatus, Spain) comprised a pull bar connected to an isometric force transducer (dynamometer). Basically, the grip strength meter was positioned horizontally, and the rats are held by the tail and lowered towards the device. The animals were allowed to grasp the bar and were then pulled backwards in the horizontal plane. The force applied to the bar just before it lost grip was recorded as the peak tension. At least three measurements were taken per rat and the results were averaged for analysis. The data are presented as g/g initial body weight.

V Statistical Analysis

Average (arithmetic mean) and standard error of the mean (SEM) were calculated for each studied variable. Statistical analysis of the data was performed by means of the Student's t-test.

Results

As can be seen in (Table 1), tumor-bearing animals experimented important decreases in body weight, carcass weight and food intake. The gastrointestinal tract was also reduced by the presence of the tumor. As previously seen in other publications in our group, formoterol treatment significantly improved these parameters (Table 1) [34, 35]. However, no effect of AR-42 treatment was observed neither on body weight nor carcass. In fact, food intake was significantly decreased by the treatment

as compared with the untreated tumor-bearing animals. The combination of formoterol and AR-42 had no effects on the above-mentioned

parameters as compared with the animals treated with the inhibitor of HDAC.

Table 1: Effects of formoterol and AR42 treatments on food intake, body weight and tumor content in tumor-bearing rats.

Parameters	Experimental groups				
	C	T	T+F	T+A	T+F+A
IBW	176 ± 5	178 ± 3	180 ± 2	176 ± 11	177 ± 4
FBW	216 ± 6	182 ± 6 ##	189 ± 6	172 ± 6	174 ± 4
ΔBW	40 ± 4	4 ± 5 ###	10 ± 5	-5 ± 5	-2,4 ± 4
Food intake	71 ± 2	63 ± 3 #	69 ± 1	51 ± 4 *	55 ± 5
Carcass	87 ± 3	78 ± 1 ##	80 ± 1	76 ± 2	75 ± 2
GIT	10527 ± 453	6756 ± 316 ###	6480 ± 296	8043 ± 699	7546 ± 417
Tumor					
Volume (mL)	-	41 ± 1	46 ± 4	40 ± 3	39 ± 4
Total cell number (10 ⁶)	-	5337 ± 539	4533 ± 386	3289 ± 458 *	3438 ± 482 *

Results are mean ± SEM for the number of animals: C (6), T (7), T+F (5), T+A (4), T+F+A (7). IBW: Initial Body Weight and FBW: Final Body Weight are expressed in g. Food intake is expressed as g/100g IBW and refers to the cumulative intake (7 days). Carcass is expressed in g/100 g IBW. GIT: Gastrointestinal Tract is expressed in mg/100 g IBW. Values that are significantly different by the Student's t-test from the control group (C) are indicated by # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and from the tumor non-treated animal group (T) are indicated by * $p < 0.05$.

C: rats without tumor; T: Tumor-bearing rats; T+F: Treated with Formoterol; T+A: Treated with AR42; T+F+A: Treated with both Formoterol and AR42.

Table 2: Effects of formoterol and AR42 treatments on muscles and adipose tissue weights in tumor-bearing rats.

Parameters	Experimental groups				
	C	T	T+F	T+A	T+F+A
Muscle Weights					
GSN	671 ± 9	568 ± 16 ###	650 ± 11 ***	569 ± 14	602 ± 13
Tibialis	216 ± 3	188 ± 5 ###	212 ± 4 ***	179 ± 7	190 ± 6
Soleus	48 ± 1	44 ± 1 #	47 ± 1 *	45 ± 2	42 ± 1
EDL	52 ± 2	44 ± 1 ###	49 ± 1 ***	42 ± 1	45 ± 1
Heart	401 ± 14	348 ± 9 ##	345 ± 18	342 ± 9	353 ± 31

Results are mean ± SEM for the number of animals: C (12), T (13), T+F (13), T+A (4), T+F+A (7). Values that are significantly different by the Student's t-test from the control group (C) are indicated by # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and from the tumor non-treated animals group (T) are indicated by * $p < 0.05$, *** $p < 0.001$.

GSN: Gastrocnemius muscle; EDL: Extensor Digitorum Longus; C: rats without tumor; T: Tumor-bearing rats; T+F: Treated with Formoterol; T+A: Treated with AR42; T+F+A: Treated with both Formoterol and AR42.

Table 2 clearly shows that tumor-bearing animals suffered an important decrease in the mass of all the individual skeletal muscles studied. Formoterol treatment significantly improved muscle weight. However, treatment with AR-42 did not show any benefits on muscle weight. On the same lines, the combination of formoterol and AR-42 showed no synergistic effects but, in fact, the positive effects alone were not seen. Since muscle mass and function are not necessarily correlated, we

decided to measure muscle force in the different experimental groups. As can be seen in (Table 3), the presence of the tumor promoted a significant decrease in grip force which was improved by formoterol as previously described [35]. Treatment of the tumor-bearing animals with AR-42 resulted in no improvement of grip force as compared with tumor-bearing animals. Similarly, the combination with formoterol and AR-42 did not show any synergistic effects.

Table 3: Effects of formoterol and AR42 treatments on grip force in tumor-bearing rats.

Parameters	Experimental groups				
	C	T	T+F	T+A	T+F+A
Grip force day 0	340 ± 11	343 ± 12	342 ± 11	286 ± 18 *	322 ± 27
Grip force day 7	426 ± 20	357 ± 14 ##	449 ± 11 ***	257 ± 12 **	377 ± 13
Δ grip force	86 ± 19	15 ± 21 #	107 ± 11 ***	-29 ± 23	55 ± 24

Results are mean ± SEM for the number of animals: C (12), T (13), T+F (13), T+A (4), T+F+A (7). Δ grip force was calculated as [(grip force day 7 - grip force day 0)/ IBW] * 100. Values that are significantly different by the Student's t-test from the control group (C) are indicated by # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and from the tumor non-treated animal group (T) are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

C: rats without tumor; T: Tumor-bearing rats; T+F: Treated with Formoterol; T+A: Treated with AR42; T+F+A: Treated with both Formoterol and AR42.

Discussion

A recent investigation suggested an involvement of histone deacetylase (HDAC) in skeletal muscle atrophy [28]. Indeed, HDAC activates the transcription factor FoxO and seems to be a sufficient mechanism for inducing skeletal muscle atrophy [28]. Bearing this in mind, previous investigations have used AR42 (an inhibitor of HDAC) for the treatment of muscle wasting associated with cancer cachexia using both the C-26 colon adenocarcinoma and Lewis lung carcinoma (LLC) models [27]. In addition, the same inhibitor has proven its efficacy as an antitumoral agent in both cancer cell lines and tumor xenografts and transgenic mouse models of pancreatic cancers [36].

Taking all this into consideration, the object of the present investigation was: i) to examine the role of AR-42 in both, tumor growth reduction and muscle wasting in a rat cancer cachexia model and ii) to examine a combination of a proven anti-muscle wasting agent (formoterol) with AR-42 in order to analyse possible synergistic effects [34, 35]. Concerning tumor growth, while formoterol treatment did not influence, AR-42 clearly and significantly decreased total tumor cell number by 38%. Interestingly, the combination of formoterol and AR-42 also resulted in a significant decrease of the tumor (Table 1). These data agree with the previous results concerning the suppression of tumor growth of the AR42 [36]. In fact, the positive effects found in previous investigations concerning a reduction of muscle wasting by AR-42 could be a consequence of the effects of the inhibition on the tumor growth [36]. Indeed, at least in pre-clinical models, any drug decreasing tumor growth is invariably associated with an improvement of muscle wasting [37-39]. Our results do not show any benefit of the inhibition on muscle mass or function, this possibly being associated with the toxicity of the inhibitor. Although the dose used in this investigation was very similar to the ones previously investigated, toxicity leads to a decreased food intake (Table 1) together with a clear anaemia induced by AR-42 (results not shown) [27, 28, 36]. Interestingly, another investigation did not find any improvements of deacetylase inhibitors in cachexia in tumor-bearing mice despite modulation of the myostatin/follistatin axis [40].

Conclusion

In conclusion, the use of AR-42 to prevent muscle atrophy associated with cancer cachexia is questionable and additional investigations are, therefore, needed.

Funding

This work was supported by a grant from the Ministerio de Economía y Competitividad (SAF2015-65589-P).

Conflicts of Interest

Each author has participated sufficiently, intellectually or practically, in the work to take public responsibility for the content of the article, including the conception, design, and for data interpretation. All authors have read and approved the final manuscript. All authors of this research have not conflict of interest related with employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding: Sílvia Busquets,

Marta Castillejo, Queralto Jové, Alina Noguera, Francisco J. López-Soriano and Josep M. Argilés declare that they have no conflict of interest.

Ethical Approval and Consent

The experimental protocol was approved by the Ethical Committee of the University of Barcelona and all animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals [31].

Author Contributions

MC: Food intake follow up; QJ: Isolation of individual muscles; AN: Tumor measurements; FLS: Laboratory supervisor; JMA: Direction; SB: Tumor implantation and follow up.

Abbreviation

EDL: Extensor Digitorum Longus
GSM: Gastrocnemius Muscle
HDAC: Histone Deacetylase
HAT: Histone Acetyltransferases
I.G.: Intragastric Administration
S.C.: Subcutaneous Administration
TB: Tumor Bearers
A: Animals treated with AR-42
F: Animals treated with Formoterol
F+A: Double-treated Animals

REFERENCES

1. Evans WJ, Morley JE, Argilés J, Bales C, Baracos V et al. (2008) Cachexia: a new definition. *Clin Nutr* 27: 793-799. [[Crossref](#)]
2. Loberg RD, Bradley DA, Tomlins SA, Chinnaiyan AM, Pienta KJ (2007) The lethal phenotype of cancer: the molecular basis of death due to malignancy. *CA Cancer J Clin* 57: 225-241. [[Crossref](#)]
3. Moses AWG, Slater C, Preston T, Barber MD, Fearon KCH (2004) Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. *Br J Cancer* 2004 90: 996-1002. [[Crossref](#)]
4. Dewys WD, Begg C, Lavin PT, Band PR, Bennett JM et al. (1980) Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group. *Am J Med* 69: 491-497. [[Crossref](#)]
5. Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM et al. (2010) Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) 'cachexia-anorexia in chronic wasting diseases' and 'nutrition in geriatrics'. *Clin Nutr* 29: 154-159. [[Crossref](#)]
6. Wolfe RR (2006) The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 84: 475-482. [[Crossref](#)]
7. Macciò A, Madeddu C, Gramignano G, Mulas C, Floris C et al. (2012) A randomized phase III clinical trial of a combined treatment for cachexia in patients with gynecological cancers: evaluating the impact

- on metabolic and inflammatory profiles and quality of life. *Gynecol Oncol* 124: 417-425. [Crossref]
8. Rogers ES, MacLeod RD, Stewart J, Bird SP, Keogh JW (2011) A randomised feasibility study of EPA and Cox-2 inhibitor (Celebrex) versus EPA, Cox-2 inhibitor (Celebrex), resistance training followed by ingestion of essential amino acids high in leucine in NSCLC cachectic patients--ACCeRT study. *BMC Cancer* 11: 493. [Crossref]
 9. Busquets S, Figueras MT, Fuster G, Almendro V, Moore Carrasco R et al. (2004) Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. *Cancer Res* 64: 6725-6731. [Crossref]
 10. Harcourt LJ, Schertzer JD, Ryall JG, Lynch GS (2007) Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul Disord* 17: 47-55. [Crossref]
 11. Busquets S, Toledo M, Sirisi S, Orpí M, Serpe R et al. (2011) Formoterol and cancer muscle wasting in rats: Effects on muscle force and total physical activity. *Exp Ther Med* 2: 731-735. [Crossref]
 12. Greig CA, Johns N, Gray C, Macdonald A, Stephens NA et al. (2014) Phase I/II trial of formoterol fumarate combined with megestrol acetate in cachectic patients with advanced malignancy. *Support Care Cancer* 22: 1269-1275. [Crossref]
 13. Eckschlagner T, Plch J, Stiborova M, Hrabeta J (2017) Histone Deacetylase Inhibitors as Anticancer Drugs. *Int J Mol Sci* 18: 1414. [Crossref]
 14. Cheng H, Xie Z, Jones WP, Wei XT, Liu Z et al. (2016) Preclinical Pharmacokinetics Study of R- and S-Enantiomers of the Histone Deacetylase Inhibitor, AR-42 (NSC 731438), in Rodents. *AAPS J* 18: 737-745. [Crossref]
 15. Chen YJ, Wang WH, Wu WY, Hsu CC, Wei LR et al. (2017) Novel histone deacetylase inhibitor AR-42 exhibits antitumor activity in pancreatic cancer cells by affecting multiple biochemical pathways. *PLoS One* 12: e0183368. [Crossref]
 16. Sargeant AM, Rengel RC, Kulp SK, Klein RD, Clinton SK et al. (2008) OSU-HDAC42, a histone deacetylase inhibitor, blocks prostate tumor progression in the transgenic adenocarcinoma of the mouse prostate model. *Cancer Res* 68: 3999-4009. [Crossref]
 17. Kulp SK, Chen CS, Wang DS, Chen CY, Chen CS (2006) Antitumor effects of a novel phenylbutyrate-based histone deacetylase inhibitor, (S)-HDAC-42, in prostate cancer. *Clin Cancer Res* 12: 5199-5206. [Crossref]
 18. Lu YS, Kashida Y, Kulp SK, Wang YC, Wang D et al. (2007) Efficacy of a novel histone deacetylase inhibitor in murine models of hepatocellular carcinoma. *Hepatology* 46: 1119-1130. [Crossref]
 19. Wei D, Lu T, Ma D, Yu K, Zhang T et al. (2018) Synergistic activity of imatinib and AR-42 against chronic myeloid leukemia cells mainly through HDAC1 inhibition. *Life Sci* 211: 224-237. [Crossref]
 20. Zhou R, Wu J, Tang X, Wei X, Ju C et al. (2018) Histone deacetylase inhibitor AR-42 inhibits breast cancer cell growth and demonstrates a synergistic effect in combination with 5-FU. *Oncol Lett* 16: 1967-1974. [Crossref]
 21. Elshafae SM, Kohart NA, Altstadt LA, Dirksen WP, Rosol TJ (2017) The Effect of a Histone Deacetylase Inhibitor (AR-42) on Canine Prostate Cancer Growth and Metastasis. *Prostate* 77: 776-793. [Crossref]
 22. Zhang S, Suvannasankha A, Crean CD, White VL, Chen CS et al. (2011) The novel histone deacetylase inhibitor, AR-42, inhibits gp130/Stat3 pathway and induces apoptosis and cell cycle arrest in multiple myeloma cells. *Int J Cancer* 129: 204-213. [Crossref]
 23. Zhang M, Pan Y, Dorfman RG, Chen Z, Liu F et al. (2016) AR-42 induces apoptosis in human hepatocellular carcinoma cells via HDAC5 inhibition. *Oncotarget* 7: 22285-22294. [Crossref]
 24. Sborov DW, Canella A, Hade EM, Mo X, Khountham S et al. (2017) A phase 1 trial of the HDAC inhibitor AR-42 in patients with multiple myeloma and T- and B-cell lymphomas. *Leuk Lymphoma* 58: 2310-2318. [Crossref]
 25. Guzman ML, Yang N, Sharma KK, Balys M, Corbett CA et al. (2014) Selective activity of the histone deacetylase inhibitor AR-42 against leukemia stem cells: a novel potential strategy in acute myelogenous leukemia. *Mol Cancer Ther* 13: 1979-1990. [Crossref]
 26. Sandri M, Lin J, Handschin C, Yang W, Arany ZP et al. (2006) PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *Proc Natl Acad Sci U S A* 103: 16260-16265. [Crossref]
 27. Tseng YC, Kulp SK, Lai IL, Hsu EC, He WA et al. (2015) Preclinical Investigation of the Novel Histone Deacetylase Inhibitor AR-42 in the Treatment of Cancer-Induced Cachexia. *J Natl Cancer Inst* 107: djv274. [Crossref]
 28. Beharry AW, Sandesara PB, Roberts BM, Ferreira LF, Senf SM et al. (2014) HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J Cell Sci* 127: 1441-1453. [Crossref]
 29. Penna F, Costelli P (2019) New developments in investigational HDAC inhibitors for the potential multimodal treatment of cachexia. *Expert Opin Investig Drugs* 28: 179-189. [Crossref]
 30. Busquets S, Serpe R, Toledo M, Betancourt A, Marmonti E et al. (2012) L-Carnitine: An adequate supplement for a multi-targeted anti-wasting therapy in cancer. *Clin Nutr* 31: 889-895. [Crossref]
 31. DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes (2010).
 32. Lu Q, Wang DS, Chen CS, Hu YD, Chen CS (2005) Structure-based optimization of phenylbutyrate-derived histone deacetylase inhibitors. *J Med Chem* 48: 5530-5535. [Crossref]
 33. Toledo M, Busquets S, Sirisi S, Serpe R, Orpí M et al. (2011) Cancer cachexia: physical activity and muscle force in tumour-bearing rats. *Oncol Rep* 25: 189-193. [Crossref]
 34. Busquets S, Figueras G, Fuster V, Almendro R, Moore Carrasco E et al. (2004) Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. *Cancer Res* 64: 6725-6731. [Crossref]
 35. Busquets S, Toledo M, Sirisi S, Orpí M, Serpe R et al. (2011) Formoterol and cancer muscle wasting in rats: Effects on muscle force and total physical activity. *Exp Ther Med* 2: 731-735. [Crossref]
 36. Henderson SE, Ding LY, Mo X, Bekaii Saab T, Kulp SK et al. (2016) Suppression of Tumor Growth and Muscle Wasting in a Transgenic Mouse Model of Pancreatic Cancer by the Novel Histone Deacetylase Inhibitor AR-42. *Neoplasia* 18: 765-774. [Crossref]
 37. Beluzi M, Peres SB, Henriques FS, Sertié RAL, Franco FO et al. (2015) Pioglitazone treatment increases survival and prevents body weight loss in tumor-bearing animals: possible anti-cachectic effect. *PLoS One* 10: e0122660. [Crossref]
 38. Jumes FMD, Lugarini D, Pereira ALB, de Oliveira A, Christoff A de O et al. (2010) Effects of *Agaricus brasiliensis* mushroom in Walker-256 tumor-bearing rats. *Can J Physiol Pharmacol* 88: 21-27. [Crossref]

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39. Nukatsuka M, Fujioka A, Saito H, Uchida J, Nakano K et al. (1996) Prolongation of survival period and improvement of cancer cachexia by long-term administration of UFT. *Cancer Lett* 104: 197-203. [[Crossref](#)]
 40. Bonetto A, Penna F, Minero VG, Reffo P, Bonelli G et al. (2009) Deacetylase inhibitors modulate the myostatin/follistatin axis without improving cachexia in tumor-bearing mice. *Curr Cancer Drug Targets* 9: 608-616. [[Crossref](#)]