

# Effect of sibutramine on regional fat pads and leptin levels in rats fed with three isocaloric diets

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**ABSTRACT.** *Aim.* The aim of the study was to investigate: a) the differential effect of the three main macronutrients on food intake, fat depots and serum leptin levels and b) the impact of sibutramine on the above parameters in rats fed *ad libitum* with three isocaloric diets. *Methods.* Three groups of male Wistar rats (n = 63) were fed with a high fat diet (HFD), a high carbohydrate diet (HCD) or a high protein diet (HPD) for 13 weeks. In the last three weeks, each group was divided into three subgroups and received sibutramine (S) either at 5 mg/kg or 10 mg/kg, or vehicle. Food intake was measured daily during the last week of the experiment; perirenal and epididymal fat and fat/lean ratio were calculated and serum leptin was assayed. *Results.* HFD-fed rats demonstrated elevated food intake and higher regional fat depots. S at 10 mg/kg decreased food intake in the HFD and epididymal fat in the HCD group. S also reduced perirenal fat in the HCD and HPD groups. Leptin levels were higher in rats fed with either the HFD or the HPD compared to those fed with the HCD. Moreover, S at 10 mg/kg decreased serum leptin levels in the HPD group. *Conclusions.* Results suggest a preferential effect of S on perirenal visceral fat and support the view that body fat loss is greater when its administration is accompanied by a HCD diet. No effect of S on leptin levels was found, besides that expected as a result of the decrease in body fat.

**Keywords:** isocaloric diets perirenal fat epididymal fat, sibutramine, leptin

Since the cloning of leptin cDNA in 1994, adipose tissue has been regarded as a novel endocrine gland that affects energy homeostasis and multiple aspects of body function. The term adipokines has been introduced to describe collectively adipose tissue-derived agents, and refers to adiponectin, leptin, resistin, visfatin, omentin and apelin [1]. Their biological actions are not solely associated with the regulation of energy intake and expenditure, but they have also been involved in the regulation of insulin sensitivity, immunity, inflammation and heart function [1, 2].

Leptin, a 144-amino acid, multifunctional protein that is considered the prototype adipokine [3], is predominantly involved in the regulation of appetite and body weight [4]. In addition, it affects insulin resistance, energy expenditure, immunity, coagulation, as well as cardiovascular and endocrine functions [5, 6]. Its levels correlate with body fat mass and are acutely suppressed by food deprivation [7]. Although leptin deficiency is associated with morbid obesity [8], the vast majority of obese individuals demonstrate normal or high serum levels accompanied by attenuated responsiveness to the adipokine, a condition known as leptin resistance [9].

Sibutramine is a selective inhibitor of the reuptake of monoamines, primarily serotonin and noradrenaline and to a lesser extent dopamine, and is used as an anti-obesity drug [10, 11]. It has a dual mode of action, as it reduces food intake by enhancing satiety and, in parallel, increases thermogenesis [12]. Moreover sibutramine attenuates the fall in metabolic rate associated with weight loss [13] and enhances locomotor activity, at least in rodents [14].

The aim of this study was to investigate:

- 1) the differential effects of the three main macronutrients on food intake, fat depots and serum leptin levels ;
- 2) the impact of sibutramine on the parameters mentioned above in rats fed three isocaloric diets *ad libitum*.

## METHODS

### Animals

Sixty-three male Wistar rats, 2 months old and of  $180 \pm 10$  g body weight were used. After acclimation for one week to constant environmental conditions (room temperature  $24 \pm 1^\circ\text{C}$ , humidity 45% and a 12/12 hour light/dark cycle-lights on at 07:00 h), rats were randomly separated into three

groups, each group receiving one of the three diets for 13 weeks. Rats from each group were individually caged and fed *ad libitum* with the respective diets: the high-fat diet (HFD), providing 40% of the energy as cottonseed oil, 8% as sucrose and 8% as casein: group HFD (n = 21); the high-carbohydrate diet (HCD), providing 68% of the energy as sucrose, 8% as cottonseed oil, and 18% as casein: group HCD (n = 21); and the high protein diet (HPD), providing 64% of the energy as casein, 8% as cottonseed oil, and 22% as sucrose: group HPD (n = 21). The diets (HFD, HCD and HPD) were isocaloric (4,24 Kcal/g), powdered and supplemented with vitamins and minerals. They were purchased, in five kilos packages, from Mucedolla s.r.l., Italy.

### Treatment procedures

After 10 weeks, rats from each group (HFD, HCD and HPD) were divided into three subgroups, (n = 7/subgroup) and sibutramine, at two different doses, or vehicle were given up to the 13<sup>th</sup> week of the experimental procedure. Sibutramine HCL was a kind gift from Abbott Laboratories (Hellas), S.A. The drug was dissolved in saline, and administered in single, daily, i.p. injections of 5 mg/kg, or 10 mg/kg (injected volume: 1 mL/kg b.w.), at 13:00, for a period of 21 days. The control subgroups received an equal volume of saline, for the same period. The subgroups that were finally created were: HFDS0, HCDS0, HPDS0, (vehicle), HFDS5, HCDS5, HPDS5 (Sibutramine-HCL – S 5 mg/kg) and HFDS10, HCDS10, HPDS10 (S 10 mg/kg) (figure 1).

Food intake was measured daily during the last week of the experiment, while body weight (BW) was measured weekly, during the whole experimental procedure.

At the end of the study, rats were euthanized under light anesthesia with a mixture of ketamine and xylazine HCL (i.m. injection of 1:0.1 mg/kg). Blood was collected immediately before death, from the vena cava inferior, and serum was separated and stored at -70°C for subsequent measurement of leptin concentrations. Gastrocnemius muscle (GM), perirenal (PF) and epididymal (EF) fat were dissected out and their weight was expressed as g/100 g of rat final body weight (BW). Moreover, fat/lean ratio (EF + PF/GM) was calculated [15]. All animals procedures were carried out in accordance with EEC-86/609 regulations under the authority of the relevant project license obtained from the Prefecture of Athens.

### Assays

Serum leptin levels were measured by enzyme-linked-immunosorbent assay, (Elisa) (Bio Vendor kit, Rat Leptin RD 291001200).

### STATISTICAL ANALYSES

Data are expressed as mean ± standard error. Significant differences between groups and subgroups were determined by one-way analysis of variance (ANOVA). In order to control for type I errors, the Bonferonni correction was used in cases of multiple comparisons. The association of two continuous variables was investigated using a partial Pearson's correlation coefficient. Statistical significance was set at 0.05. All p values reported are two-tailed and analysis was conducted using SPSS 13.0.

### RESULTS

#### Mean food intake

The mean consumption of each subgroup receiving the respective diet and sibutramine is shown in figure 2.

A significant increase in mean food intake was observed in HFDS0 compared to HCDS0 (p = 0.05) and HPDS0 (p = 0.02) (vehicle subgroups), as well in HFDS5 compared to HCDS5 (p = 0.004) and HPDS5 (p = 0.001) (S 5 mg/kg subgroups). No differences between the HFDS10, HCDS10 and HPDS10 were found (F<sub>2,18</sub> = 3.427, p = 0.055) (S 10 mg/kg subgroups).

Mean food intake was significantly different between the subgroups of the HFD group; it was significantly lower in HFDS10 compared to subgroups HFDS0 and HFDS5 (p = 0.018 and p = 0.026 respectively). Conversely, mean food intake was not statistically different between the subgroups of the HCD group (F<sub>2,18</sub> = 2.147, p = 0.146) and the HPD group (F<sub>2,18</sub> = 0.456, p = 0.641).

#### Perirenal fat (PF)

PF tissue weight was significantly higher in all subgroups of the HFD group, compared to those of the HCD and HPD groups (p < 0.001).

The PF tissue weight was significantly different between the subgroups of the HCD and HPD groups; it was signifi-

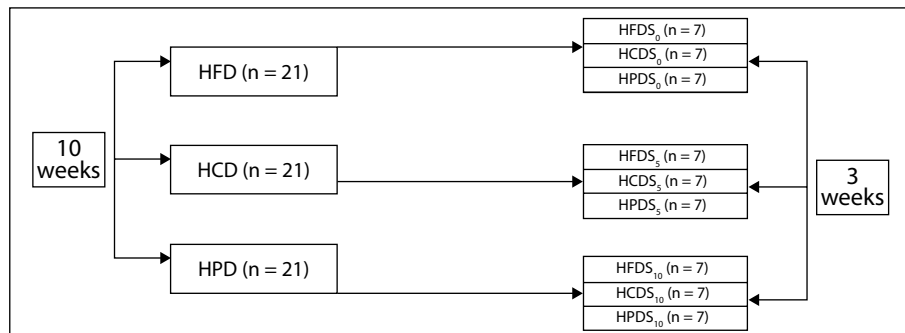
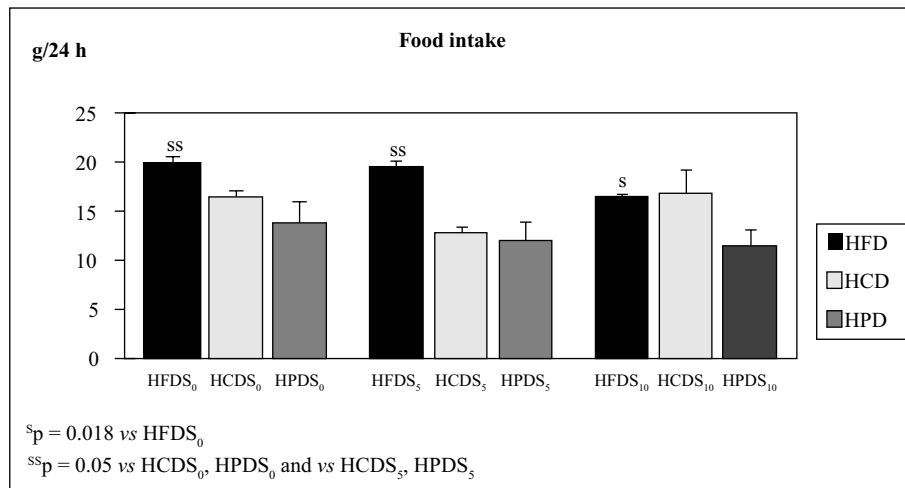


Figure 1

Groups and subgroups of rats according to their diet and drug treatment.

**Figure 2**

Mean food intake of each group of rats fed with a HFD, HCD, or HPD after vehicle, or sibutramine treatment.

cantly lower in HCDS5 and HCDS10 subgroups compared to HCDS0 ( $p = 0.048$  and  $p = 0.003$  respectively), as well as in the HPDS10 subgroup ( $p = 0.046$ ) compared to HPDS0. Conversely, the PF tissue weight was not statistically different between the subgroups of the HFD group ( $F_{2,18} = 0.835$ ,  $p = 0.45$ ) (figure 3).

#### Epididymal fat (EF)

EF tissue weight was significantly higher in the HFDS0 subgroup compared to HCDS0 ( $p = 0.004$ ) (vehicle subgroups), in HFDS5 compared to HCDS5 and HPDS5 ( $p < 0.001$ ), as well as in HFDS10 compared to HCDS10 and HPDS10 ( $p < 0.001$ ).

The EF tissue weight was significantly different between the subgroups of the HPD group. It was significantly lower in the HPDS10 subgroup ( $p = 0.042$ ) compared to HPDS0. Conversely, the EF tissue weight was not statistically different between the subgroups of the HFD group ( $F_{2,18} = 0.224$ ,  $p = 0.802$ ), or HCD group ( $F_{2,18} = 0.695$ ,  $p = 0.512$ ) (figure 4).

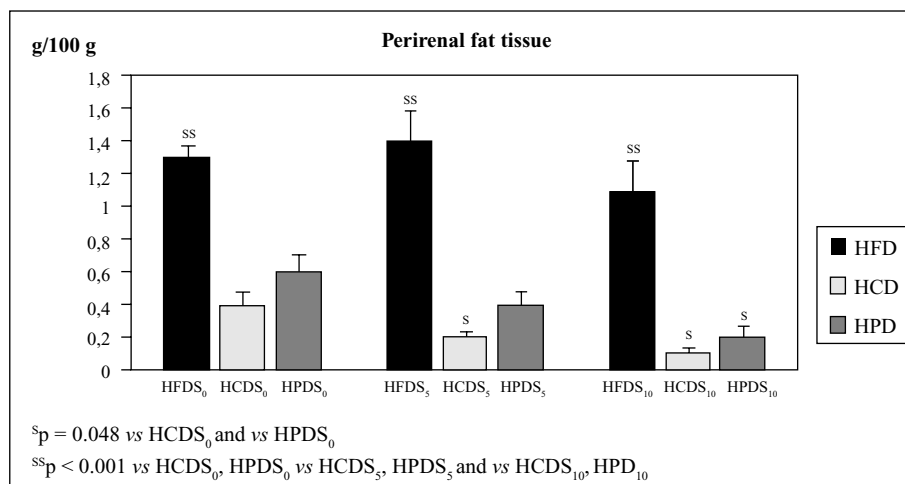
#### Fat/lean ratio

The fat/lean ratio was significantly higher in HFDS0 compared to HCDS0 ( $p < 0.001$ ) and to HPDS0 ( $p = 0.038$ ), in HFDS5 compared to HCDS5 and to HPDS5 ( $p < 0.001$ ), and in HFDS10 compared to HCDS10 and to HPDS10 ( $p < 0.001$ ).

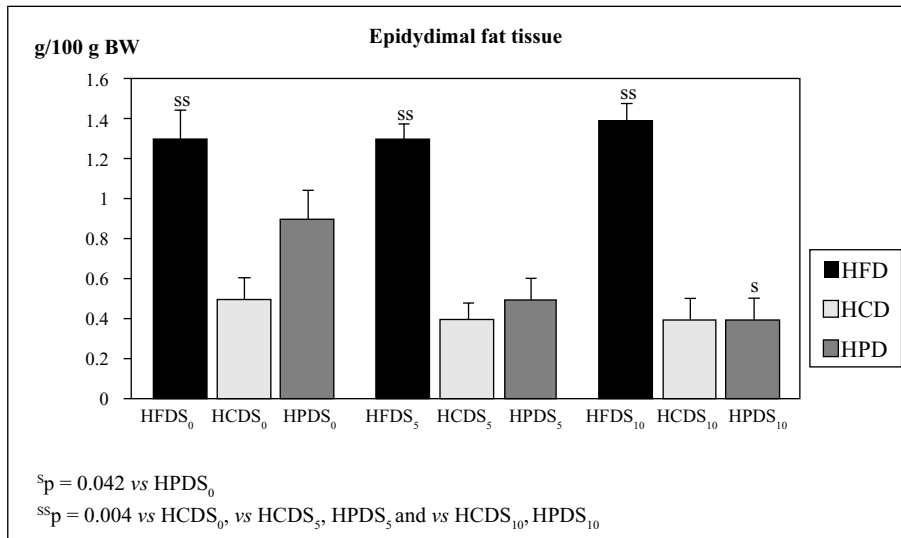
The ratio was significantly different between the subgroups of the HCD and HPD groups. It was significantly lower in the HCDS10 subgroup compared to HCDS0 ( $p = 0.05$ ), as well as in the HPDS10 subgroup ( $p = 0.033$ ) compared to HPDS0. Conversely, the fat/lean ratio was not statistically different between the subgroups of the HFD group ( $F_{2,18} = 0.426$ ,  $p = 0.659$ ) (figure 5).

#### Serum leptin

Serum leptin levels were significantly higher in HFDS0 ( $p = 0.003$ ) and in HPDS0 ( $p = 0.025$ ) compared to HCDS0; in HFDS5 compared to HCDS5 and to HPDS5 ( $p < 0.001$ ), and in HFDS10 compared to HCDS10 ( $p < 0.001$ ) and to HPDS10 ( $p = 0.001$ ).

**Figure 3**

Effects of each diet and sibutramine treatment on perirenal fat tissue weight of rats.



**Figure 4**

Effects of each diet and sibutramine treatment on epididymal fat tissue weight of rats.

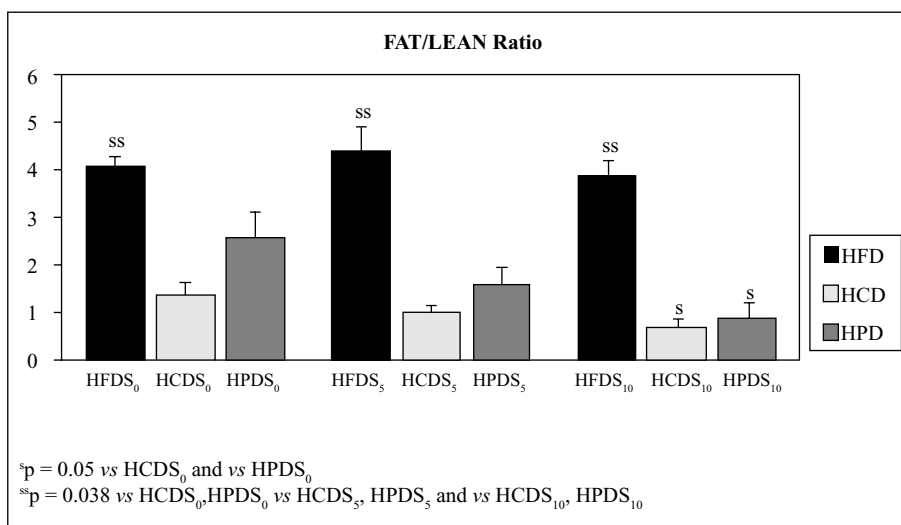
Serum leptin levels were significantly different between the subgroups of the HPD group. They were significantly lower in the HPDS10 subgroup ( $p = 0.042$ ) compared to HPDS0. Conversely, serum leptin levels were not statistically different between the subgroups of the HFD group ( $F_{2,18} = 2.563$ ,  $p = 0.105$ ), or the HCD group ( $F_{2,18} = 0.473$ ,  $p = 0.63$ ) (figure 6).

**DISCUSSION**

A high fat diet is accompanied by hyperphagia, increased meal sizes and caloric intake, obesity and increased regional fat depots [16-27]. In our study, food consumption, as well as regional fat pads, were elevated in rats receiving a HFD compared to those receiving a HCD or a HPD. The effect of a HFD was not due to the difference in energy density, since all rodents consumed isocaloric diets. Leptin levels [21, 22] and expression [25, 28] are elevated in diet-induced obesity. In our study, leptin levels were

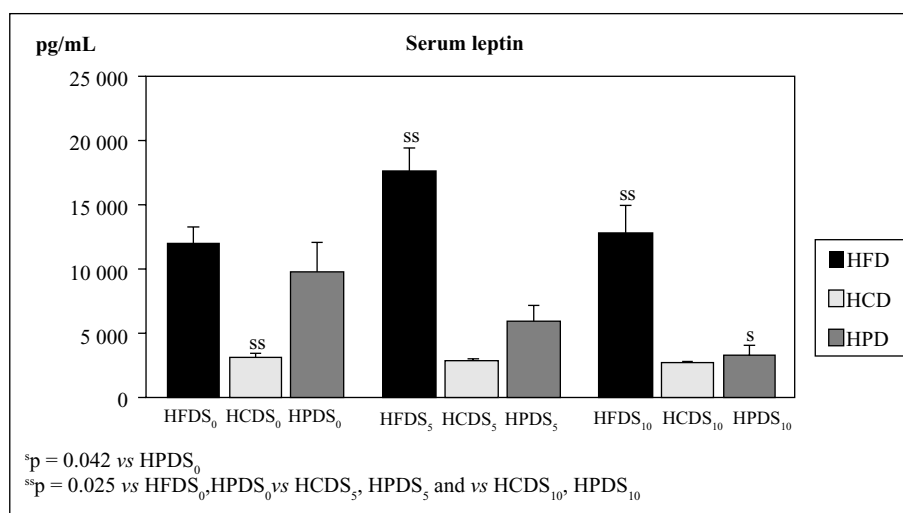
higher in the HFD group, regardless of the use of sibutramine. These findings are in agreement with those of previous studies and are indicative of the development of leptin resistance in chronically fat-fed animals [21, 22].

Administration of sibutramine at 10mg/kg significantly reduced food intake in the HFD group. This observation is in agreement with data from previous studies [29-31]. The anorectic effect of the drug has been attributed to the enhancement of POMC expression [32] and the downregulation of orexin [33]. These changes not only reset central pathways to a new, lower, basal level, but they also attenuate their responsiveness to further reductions in body energy stores. It is noteworthy that sibutramine at 10 mg/kg, reduced mean food intake to values similar to those observed in rats receiving a HPD or a HCD. It seems that the drug selectively diminishes the hyperphagic effect of high fat feeding without affecting the dietary behavior of animals consuming carbohydrate- or protein-based diets. Although it is known that sibutramine exerts a greater anorectic effect in animals fed a high fat diet rather than a



**Figure 5**

Effects of each diet and sibutramine treatment on fat/lean ratios.



**Figure 6**

Serum leptin levels of rats fed with a HFD, HCD and HPD, with and without sibutramine treatment.

standard laboratory diet [15], our study is the first to directly compare and link macronutrient composition with the response to the drug. Moreover, this is the first study assessing the anorectic effect of sibutramine on rats fed a high carbohydrate or a high protein diet.

The lack of effect of sibutramine on animals fed the HPD or HCD can be either attributed to the insensitivity of these animals to the anorectic effects of the drug or to the rapid development of tachyphylaxis that has been associated with its use [30, 32]. Unfortunately, there are no published reports of sibutramine administration in rats fed high protein or high carbohydrate diets. Moreover, the measurement of food intake during the last week of the experiment only, did not allow us to confirm the development of tachyphylaxis.

Although the anorectic effect of sibutramine is considered significant, the ultimate criterion for estimating its clinical efficacy is its ability to reduce regional fat pads. Our data demonstrate a significant effect on body weight in HPD- and HCD-fed rats. This is not due to the restriction of food intake, and can be attributed to an increased metabolic rate associated with drug use. Indeed, sibutramine and its metabolites enhance thermogenesis [12, 32, 34, 35] and locomotor activity [14, 31], thus increasing mean energy expenditure. On the other hand, the anorectic effect of sibutramine on high fat-fed rats did not result in weight loss, despite the significant reduction in body weight increasing rate during the last three weeks of the experimental procedure [36]. Similar results have also been observed previously [29]. However, in our study, the rats not only received higher doses of sibutramine (10 mg/kg rather than 3 mg/kg), but also for a longer period (three weeks rather than one week). These results indicate that animals fed a high fat diet are more resistant to the lipolytic effects of sibutramine, a finding that requires further investigation.

Although the loss of fat mass is always considered beneficial, visceral fat reduction is considered to be of great significance in attenuating the metabolic and cardiovascular burden associated with obesity. Since the preferential effect of sibutramine on visceral fat has been demonstrated in clinical and experimental studies [37-40], we investigated whether the drug has a selective effect on the different components of visceral fat. We examined perirenal and epididymal fat on the basis of previous observations show-

ing inconsistencies between the two depots in their response to various stimuli [41-45]. Moreover, there are limited data that compare the effects of sibutramine on different visceral adipose tissue depots [46].

According to previous studies, sibutramine at 7 mg/kg for four weeks significantly reduces perirenal fat [46] in rats fed a standard laboratory diet, while the data regarding the epididymal adipose tissue are conflicting [46-48]. In our study, perirenal fat was significantly reduced in both HPD and HCD groups (75% in the HCD group and 67% in the HPD group compared to the respective vehicle subgroups). On the other hand, epididymal fat was significantly reduced in the HPD group only, and at a magnitude lower than that observed for the perirenal fat [20% in the HCD group (non-significant) and 50% in the HPD group compared to the respective vehicle subgroups]. Our data expand the observations of Giordano *et al.*, by highlighting the importance of nutrition [46]. Indeed, the perirenal fat seems to be more susceptible to the lipolytic effects of sibutramine, and this effect is more prominent in rats fed diets low in fat.

Despite the fact that both leptin and sibutramine affect appetite, there are limited data regarding the possible interaction between these two agents. In our study, sibutramine treatment did not affect leptin levels in the HFD group. However, in the HPD group, the fall in leptin levels was proportional to that for the perirenal fat and the fat/lean ratio. Of particular interest is the lack of effect of sibutramine on leptin levels in the HCD group, despite the significant reduction in both fat/lean ratio and PF, a result that requires further investigation. Perhaps, the reduction in perirenal fat was not sufficient to produce a significant change in serum leptin.

In conclusion, we failed to demonstrate any significant effect of sibutramine on serum leptin levels, regardless of the drop in adipose tissue mass in the high protein-fed rats, a finding that is in accordance with previous studies in rodents [29, 32]. It is therefore proposed that any change observed in leptin levels in our study, was solely due to the loss of adipose tissue mass and did not represent a direct effect of sibutramine on leptin.

To summarize, our study demonstrated the significant effect of sibutramine on visceral fat in rodents, with a preference for perirenal fat. Moreover, it has shown that body

fat loss is greater when sibutramine treatment is accompanied by carbohydrate- or protein-rich diets, rather than diets with fat as the main energy source. Finally, we were unable to demonstrate any effect of sibutramine on leptin levels, besides that expected as the result of the decrease in body fat.

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