Metabolic Activity of Human Chorionic Gonadotropin (hCG) on Glycemia and Leptinemia in Experimental Animals Fed a Cafeteria Diet

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Running title: hCG Affects Glycemia and Leptinemia in Animals

ABSTRACT

Objectives: To elucidate the relationship of hCG administration to glycemia, Non Esterified Fatty Acids (NEFA), leptin and adiponectin levels on experimental animals previously submitted to a cafeteria diet, and then to a Low Calorie Diet (LCD). **Design:** Forty-one rats were selected (21 females, 20 males) and divided into seven (0-6) groups. Animals from groups 1 to 6 were fed a "cafeteria diet" with a mean energy content of 10% protein, 30% carbohydrate and 60% fat. Animals from group 0 were fed the standard laboratory diet. After the fattening period, animals from groups 1 to 6 were submitted to a restricted diet consisting of one-third the average daily intake for rats. hCG was administered for five weeks according to a specific protocol. The effects of hCG treatment were evaluated using analysis of variance (ANOVA). **Results:** These assessments were compared: (1) glycemia, adiponectins, leptins and non-esterified fatty acids (NEFA); (2) weight; (3) formulation effect; and (4) dose effect. Differences in leptins were observed between the Control group and Injectable A (p=0.026), Intrarectal Suspension A (p=0.20), Intrarectal Suspension B (p<0.001), and Intrarectal Suspension C (p<0001) groups. In all cases, the average values were higher for the control group. Significant differences were found in the groups treated with Injectable B, Intrarectal Suspension B (p=0.025) and Intrarectal Suspension C (p=0.037). Groups receiving Intrarectal Suspension B or C showed significantly lower mean leptin values. Differences in glycemia were detected between the Control group and Intrarectal Suspension A (p=0.021) and Intrarectal Suspension B (p=0.020) groups. Groups treated with Intrarectal Suspension A or B showed lower mean blood glucose values. **Conclusions**: Results show the activity of hCG (both urinary and recombinant) on glycemia and leptins levels in

38 experimental animals in different formulations, but specifically when administered
39 intrarectal. hCG administration significantly decreased blood sugar and leptin levels,
40 whereas adiponectins were only relatively sensitive to hCG treatment.

41 **Keywords:** Human chorionic gonadotropin (hCG); Leptins; Glycemia; Adiponectin.

INTRODUCTION

Human chorionic gonadotropin (hCG) was discovered in 1927 by Ascheim and Zondek in the urine of pregnant women and was used for the treatment of conditions such as infertility, cryptorchidism, and obesity [1-3]. Several extragonadal therapeutic actions have been attributed to hCG, including (but not limited to) Kaposi sarcoma, glaucoma and BPH (Benign Prostatic hypertrophy). One of the most controversial indications was its use in the management of obesity, until a series of double blind studies conducted in humans concluded that weight loss under hCG was no better than placebo. The standard administration route was intramuscular. Its efficacy in obesity treatment was debated for years until some studies found it was not useful for treating this condition [4-8]. In 1987, Vogt and Belluscio published a study concluding the hCG protocol originally designed by Simeons [3] for obesity treatment was a suitable and safe approach to manage this condition [9]. The authors also reasserted the role of the hypothalamus as a possible intermediate organ for the lipolytic action of hCG [9].

In 1999, Belluscio et al. worked on a modification of the hCG administration route as a strategy to modify its biological activity. They investigated the sublingual route, proposed a change in the administered dose, and in a double-blind study demonstrated the pharmacological activity of hCG in the reduction of adipose tissue total mass in volunteer subjects [10 11].

Leptin was discovered in rats in 1994. Subsequently, the human Ob gene was located on chromosome 7. It is a cytokine that plays a key role in the regulation of energy balance. It is believed to act as a lipostate: when the amount of fat stored in adipocytes increases, leptin is released into the bloodstream and results in a negative feedback signal that acts

on the hypothalamus to inhibit appetite. When adipose tissue mass increases beyond the point of equilibrium, the synthesis and secretion of leptin increases. This, in turn, stimulates several compensatory effects in the hypothalamus such as: anorectic peptide production and suppression of orexigenics, increase of energy expenditure, increase of basal metabolic rate and body temperature, and modification of the hormonal balance point, thereby reducing lipogenesis and increasing lipolysis [12-19]. The regulation of leptin secretion is associated with variations in body mass and insulin-stimulating effects. However, many obese people have high serum concentrations of leptin or resistance to it, indicating that other molecules such as ghrelin, serotonin, cholecystokinin and neuropeptide Y also have an effect on satiety and contribute to body weight regulation [20-25]. The molecular basis of leptin resistance is poorly understood; although the most accepted hypothesis is its inability to cross the blood brain barrier or the result of defects in the leptin receptor [26].

Adiponectin is a peptidic hormone abundantly expressed in mature adipocytes that circulate in high concentrations in plasma. Adiponectin expression decreases in all processes related to inflammation and insulin resistance such as obesity and diabetes mellitus. Plasma adiponectin decreases before the onset of obesity and insulin resistance in primates, suggesting that hypoadiponectinemia contributes to the pathogenesis of these diseases. Adiponectin levels increase when insulin sensitivity improves, either due to the reduction in body weight or to treatment with insulin sensitizing drugs [27].

The purpose of this study was to determine by plasma biochemistry analysis the metabolic activity of different hCG formulations, either urinary or recombinant, as well

as its relationship to glucose, NEFA, adiponectin and leptin metabolism, and to assess its safety (particularly gonadal) through histological observations of target organs.

SUBJECTS AND METHODS

The study was conducted between December 12, 2008 and June 15, 2009 at the BIO FUCAL S.A. Center located at Acceso Norte km. 42.5, Del Viso, Buenos Aires, Argentina, and sponsored by Daniel Belluscio MD. Forty-one rats (Rattus norvegicus, Sprague Dawley strain) were selected comprised of 21 females and 20 males and divided in seven (0-6) groups. Animals in groups 1 to 6 were fed a hypercaloric and highly palatable cafeteria diet [28], in contrast to animals from group 0, which continued with the standard laboratory diet. The amount of food provided with this diet was "ad libitum" and extended from December 12, 2008 (day 0 of treatment) to January 27, 2009. After the fattening period, animals in groups 1 to 6 were subjected to a restricted diet consisting of one-third of the average daily intake of balanced food for rats, calculated separately for both males and females.

hCG administration lasting five weeks was performed according to the following protocol. Group 0 received no medication or diet and continued with the standard diet throughout the course of the study. Group 1 was submitted to a hypocaloric diet without hCG administration. Group 2 was submitted to a hypocaloric diet and received 125 International Units (IU) of hCG (urinary, Massone Laboratories, Buenos Aires, Argentina) dissolved in normal saline (NaCl 0.9%), administered intramuscularly and daily, including Sundays (Injectable A). Group 3 was submitted to a hypocaloric diet and received 125 IU of r-hCG (recombinant, Ovidrel®, Serono Laboratories, Buenos Aires,

Argentina) dissolved in normal saline (0.9% NaCl), administered intramuscularly and daily, including Sundays (Injectable B). Group 4 was submitted to a hypocaloric diet and received 300 IU of hCG (urinary, Massone Laboratories, Argentina) in intrarectal emulsion containing 8 mg/ml of cyclodextrin (Laboratory Roquette Freres, Lestrem, France) as enhancer, daily, including Sundays (Intrarectal Suspension A). Group 5 was submitted to a hypocaloric diet and received 300 IU of hCG (urinary, Massone Laboratories, Argentina) in intrarectal emulsion containing 16 mg/ml of cyclodextrin (Laboratory Roquette Freres, France) as enhancer, daily, including Sundays (Intrarectal suspension B). Group 6 was submitted to a hypocaloric diet and received 300 IU of r-hCG (recombinant, Ovidrel®, Serono Laboratories) as intrarectal emulsion containing 8 mg/ml of cyclodextrin (Laboratory Roquette Freres, France) as enhancer, daily, including Sundays (Intrarectal Suspension C).

Injections were administered using 1 ml syringes and 16 x 5needles to the rear limbs between the semimembranosus and semitendinosus muscles, alternating one member per day. For intrarectal administration of the suspensions, the same syringes were used attached to an oesophageal probe for oral administration. The emulsion was deposited over the entire rectal surface, proximal to distal, keeping the anus closed for 1 minute. Both suspensions and injections were renewed every week and kept refrigerated at all times to ensure their biological activity.

Observations were systematically recorded on each treated animal once a day throughout the duration of the trial. Body weight was assessed on days 0, 3, 6, 14, 21, 25, 33, 39 (beginning of the treatment), 46, 53, 63, 77 and 82. The following serological determinations were assessed in each group at both baseline (day 39) and on the final day

(82), pre- and post- treatment, respectively: Glycemia (g/L) (Colorimetric end-point technique Autoanalyzer Hitachi 902 Wiener); adiponectin (ng/mL) (Rat adiponectin ELISA kit-ELISA manual- Catalog Nº K4903-100-Lot40203-Biovision Incorporated); leptin (ng/mL) (Mouse Leptin-Quantikine Immunoassay-ELISA-Lot 259828-Catalog Nr. MOBOO R&D Systems) and NEFA (mEq/L) (Mouse Non-ester Fatty Acid (NEFA) ELISA Kit Product No.: CSB-E13618m-CUSABIO BIOTECH Co). Regarding the safety of hCG, histological evaluation of a general necropsy was performed. The following organs and tissues were removed to perform the pertinent histopathological studies: brain (half in buffered formaldehyde at 5% and half-frozen at -20° C), ovaries (formaldehyde 5%) and testicles (5% formalin).

Statistical methodology

The effects of hCG treatment were evaluated using analysis of variance (ANOVA). The Kolmogorov-Smirnov test was also used to assess normality of distributions. Nonparametric analysis of variance was used to compare weights between treatments at the beginning and end of treatment. Descriptive analysis of adverse events was performed. SPSS® software V. 11.5 (Cary, IN, USA) was used to assess the determinations.

RESULTS

We compared basal and final determinations as follows.

General

Basal determinations

Figures 1 A-D show baseline results (before treatment) in the seven groups. To estimate their homogeneity, values were compared among the six groups submitted to high-calorie diets. No significant differences were found between groups: leptin (Fig. 1A), p=0.056; glycemia (Fig. 1B), p=0.291; adiponectin (Fig. 1C), p=0.364; and fatty acids (Fig. 1D), p=0.722.

Final determinations

Figures 2 A-D show final results (post treatment) in the seven groups. No significant differences were observed in adiponectin (F=2,130, p=0.076) (Fig. 2C) or fatty acids (F=1,056, p=0.408) (Fig. 2D), but statistically significant differences were observed in leptin (F=7,066, p<0.001) (Fig. 2A) and glucose (F=3,012, p=0.018) (Fig. 2B). Differences in leptin were observed between the Control group and the following groups: Injectable A (p=0.026), Intrarectal Suspension A (p=0.20), Intrarectal Suspension B (p<0.001) and Intrarectal Suspension C (p<0001). In all cases, the average values were higher for the Control group. Significant differences were also found in the group treated with Injectable B and in the Intrarectal Suspension B (p=0.025) and Intrarectal Suspension C (p=0.037) groups. Groups receiving Intrarectal Suspension B or C showed significantly lower mean leptin values. Differences in glycemia were detected between the Control group and the Intrarectal Suspension A (p=0.021) and Intrarectal Suspension B (p=0.020) groups. Groups treated with Intrarectal Suspension A or B showed lower mean blood glucose values.

Treatment effect

Differences were first assessed between the Control group (Group 0), the group that was submitted to the hypocaloric diet (Group 1), and groups treated with hCG (Groups 2-6) (treatment effect).

Leptin

Significant differences were found in leptins among the treatments groups (F=9,694, p<0.001). The average value in the Control group was 3.05, 1.92 in the group treated only with hypocaloric diet, and 1.12 in groups treated with hCG. The most significant differences were found between the Control group and groups treated with hCG (p<0,001), while no significant differences were found between the two groups that did not receive hCG.

Glycemia

Significant differences were also observed in plasmatic glucose final values (F=8,099, p=0,001). The average value in the Control group was 1.78, 1.23 in the group treated with hypocaloric diet, and 1.15 in the groups treated with hCG. This difference is significant when comparing the Control group to the groups treated with hCG (p=0,001). Even though adiponectin plasmatic results were higher in the groups treated with hCG, differences were not statistically significant (F=1,388, p=0.262). The average value in the Control group was 2.69, 4.12 in the group treated with hypocaloric diet, and 5.80 in the groups treated with hCG. Statistically significant differences were not found in fatty acids (F=0.763, p=0.473). The average value for the Control group was 0.97, 0.85 in the hypocaloric diet group, and 0.90 in the groups treated with hCG.

Dose effect

198 To assess the effect of the administered dose, groups were matched as follows: Control
199 with standard diet, Control with hypocaloric diet, Injection A/Intrarectal Suspension A,
200 Injectable B / Intrarectal Suspension C, Intrarectal Suspension B.

201 ***Leptin***

202 Significant differences in leptin were observed between the groups (Brown-Forsythe
203 5.473; p=0.009). The highest average values were recorded in the group that received the
204 standard diet (3.05). Values were lower (1.92) in the group treated with hypocaloric diet,
205 and even lower in the groups receiving hCG. Among those groups, the lowest mean
206 values were recorded in animals receiving Intrarectal Suspension B. Differences were
207 significant between the Control group and the groups receiving Injectable A/Intrarectal
208 Suspension A (1.28, p=0.010), groups that received Injectable B/ Intrarectal Suspension
209 C (1.30, p=0.012), and groups that received Intrarectal Suspension B (0.47, p<0.001).

210 ***Glycemia***

211 Significant differences were observed in blood glucose between groups (F=4,078,
212 p=0.008). Animals from the Control group showed higher average blood glucose values
213 (1.78). A reduction in average values was observed in the group treated with hypocaloric
214 diet (1.23) and in all subjects receiving hCG. When comparing animals under treatment,
215 the lowest mean average values were observed in those receiving Intrarectal Suspension
216 B (0.90). Significant differences were observed between the Control group and the group
217 receiving Injectable A/Intrarectal Suspension A (1.05, p=0.016), the group receiving
218 Injectable B/Intrarectal Suspension C (1.05, p=0.018), and the group receiving Intrarectal
219 Suspension B (p=0.009).

Formulation effect

To estimate the effect of the administered formulation, groups were matched and analyzed as follows: Control with standard diet, Control with hypocaloric diet, subjects with Injectable A/Intrarectal Suspension, A/Intrarectal Suspension B, and subjects with Injectable B/Intrarectal Suspension C.

Leptins

Significant differences in leptin levels were observed between the groups (Brown-Forsythe 4978; p=0.020). The highest average values (3.05) were observed in the Control group with standard diet. Values were lower (1.92) in the Control group with hypocaloric diet and in the groups receiving hCG. When comparing groups, the lowest mean values (1.01) were observed in animals receiving Injectable A/Intrarectal Suspension A/Intrarectal Suspension B. Statistically significant differences were found when comparing the Control group with standard diet and animals receiving Injectable A/Intrarectal Suspension A/Intrarectal Suspension B (p=0,001) and Injectable B/Intrarectal Suspension C (1.30, p=0.009).

Glycemia

Significant differences were observed when comparing blood glucose levels between the groups (F=5.307, p=0.004). The highest average values (1.78) were detected in the Control group that received the standard diet, and values decreased in the Control group treated with the hypocaloric diet (1.23) and in groups receiving hCG (1.00 and 1.05, respectively). Differences were significant between the Control group with the standard

diet and the groups receiving Injectable A/Intrarectal Suspension A/Intrarectal Suspension B (p=0.003) and Injectable B/Intrarectal Suspension C (p=0.010).

Pharmaceutical formulation effect

To estimate the different effects of the pharmaceuticals formulations, groups were split as follows: Control group with standard diet, Control group with hypocaloric diet, a group with Injectable A/B and a group with Intrarectal suspension A/B/C.

Leptin

Significant differences were observed in leptins between groups (Brown-Forsythe 7.398; p=0.008). The highest average values (3.05) were recorded in the Control group with the standard diet. In the Control group with the hypocaloric diet, the observed value (1.92) was decreased and further reductions were observed in the groups receiving hCG. Among the groups receiving treatment, lower average values (0.75) were found in the intrarectal suspension A/B/C groups. Differences were significant between the Control group with standard diet, the group receiving Injectable A/B (1.72, p=0.041), and the group receiving Intrarectal suspension A/B/C ($p < 0.001$). Differences were also significant between the groups with the hypocaloric diet and the Intrarectal suspension group (p=0.040), and the Injectable and Intrarectal suspension groups (p=0.034).

Glycemia

Significant differences were observed in blood glucose levels among the groups (Brown-Forsythe F=5,667, p=0.003). Animals with the standard diet showed higher average blood glucose values (1.78). Mean values dropped (1.23) in the group treated with hypocaloric diet and in all groups receiving hCG. Among the treated groups, the lowest

mean values (0.97) were found in those receiving intrarectal suspension A/B/C. Significant differences were found between the Control group with standard diet, groups receiving Injectable A/B (1.11, p=0.019), and groups that received the Intrarectal suspension A/B/C (p<0.001).

Weight assessment

Figure 3 shows modifications in the mean weight of the seven groups.

Treatment effect

Significant differences were found between the groups regarding weight modifications (F=13,254, p<0.001). The average percentage variation for the standard diet group was 0.4% (CI 95%; 8.8, 9.6). Results for the group with the hypocaloric diet were -24.7% (CI 95%; 29.9, 19.4) and for hCG-treated groups they were -16.8% (CI 95%; -20.3, -13.3). Differences were significant in all three comparisons: the Control group with standard diet *vs*. the hypocaloric diet group (p<0,001); the Control group with standard diet *vs*. hCG-treated groups (p<0,001); and the Control group with hypocaloric diet *vs*. hCG-treated groups (p<0.001).

Dose effect

To assess the effect of the administered dose, groups were matched as follows: the Control group with standard diet, the Control group with hypocaloric diet, the groups with Injectable A/Intrarectal Suspension A, Injectable B/ Intrarectal Suspension C, and Intrarectal Suspension B. Significant differences were observed in weight percent change among groups between day 39 (baseline; before treatment, after cafeteria diet) and day 82 (Brown-Forsythe=10,394, p=0 <0.001). Significant differences were also observed when

comparing the Control group under the standard diet (average percentage variation 0.4; CI 95%; 8.8, 9.6) *vs*. the Control group with the hypocaloric diet (-24.7, CI 95% -29.9, -19.4) (p<0.001); *vs*. the Injectable A/Intrarectal Suspension A group (-18.1, CI 95% -25.1, -11.2) (p=0,001); and *vs*. the Injectable B / Intrarectal Suspension C group (-18.3, CI 95% -23.0, -13.7) (p=0,001). There was also a significant difference between the Control group with hypocaloric diet and the Intrarectal suspension B group (-8.7, CI 95%;-16.7, -0.6) (p=0.037).

Formulation effect

To assess the effect of the administered formulation, groups were analyzed as follows: the Control group with standard diet, the Control group with hypocaloric diet, and the Injectable A/Intrarectal suspension A/ Intrarectal suspension B, Injectable B/ Intrarectal suspension C groups. Significant differences in average weight percentage variations were observed between day 39 (baseline) and day 82 between the groups (Brown-Forsythe=11.201; 0.4-8.8, 9.6 p=0<0.001). Significant differences appeared when comparing the Control group with the standard diet (average percentage variation 0.4; CI 95% CI; -8.8, 9.6) *vs*. the Control group with hypocaloric diet (-24.7, CI 95%; -29.9, -19.4) (p<0.001); *vs*. the Injectable A/Intrarectal suspension A/Intrarectal suspension B group (-15.6, CI 95%; -21.2, -10.0) (p=0.004); and *vs*. the Injectable B/Intrarectal suspension C (-18.3, CI 95% -23.0, -13.7) (p=0,001) group.

Histopathology

Significant morphological changes are summarized in Tables 1, 2 and 3.

DISCUSSION

Leptin plays a key role in the regulation of energy metabolism. In disorders such as overweight and obesity, it is often elevated in plasma, suggesting that resistance to its action results in an impairment of the regulation of adipose tissue metabolism. Weight gain also determines the presence of hyperglycaemia, a metabolic situation that clearly aggravates the underlying pathology (obesity). In this study, it was possible to observe relevant differences about the effects of leptins. While the Control group with the standard diet started the study with significantly lower mean values, the achieved reduction was significantly less. Significant reductions in leptins were observed in the Control group with hypocaloric diet and in the Injectable A and B groups. At the end of the study, leptin results continued to be significantly different among some groups. The Control group with the standard diet showed higher average values, while the Intrarectal suspension B and C groups showed the lowest values.

In addition, significant differences were also observed in mean blood glucose results. The Control group with the standard diet achieved the highest average values; higher than those of the groups treated with intrarectal suspension A or B. The Control group with the standard diet showed mean leptin and blood glucose values significantly higher than the groups treated with hCG. Moreover, no significant differences were found between the values of the Control group that received a standard diet and the Control group with the hypocaloric diet.

Adiponectins and fatty Acids are not very sensitive to treatment when evaluating different doses and formulations. However, it was observed that, in relation to adiponectin, its values were elevated in animals receiving the hypocaloric diet, and even more so in the groups treated with hCG. Leptin and glucose levels were sensitive to

treatment. Leptin levels were significantly higher in the Control group, were decreased in the hypocaloric diet group, and even more decreased in the animals that received hCG. When comparing analysis per dose, the group treated with intrarectal suspension B showed the lowest values: the Injectable A/Intrarectal suspension A/Intrarectal suspension B groups showed the lowest levels in the analysis of the formulation, and the Intrarectal suspension A/B/C groups showed the lowest levels in the analysis of the pharmaceutical form. It is emphasized that no significant differences were observed between the groups that did not receive hCG. A similar effect was observed regarding glycemia. Treated groups showed significantly lower mean values in animals treated with Intrarectal suspension B (per dose analysis), in the animals treated with Injectable A/Intrarectal suspension A/Intrarectal suspension B (in the analysis of formulation), and in the animals treated with Intrarectal Suspension A/B/C (in the analysis of pharmaceutical form).

These results demonstrate the activity of hCG (both urinary and recombinant) on glycemia and leptins levels in different formulations, but especially when administered intrarectal. Similarly to human studies performed by one of the authors (DOB), this activity did not correlate with a greater weight loss when compared to a population submitted to a standard hypocaloric diet. This result could either be attributed to the small number of animals in each group or it may also indicate a possible hCG effect on body composition, thereby favouring an increase in the lean mass component without modifying the total body weight. In addition, no significant adverse clinical effects were observed with the suprapharmacological doses administered (up to 400 times the dose/kg of body weight administered in humans).

These findings confirm the results from former studies in humans that show that weight loss under hCG is no different when compared to placebo-treated individuals. However, according to the authors, this is the first report that shows that hCG has a definite action on leptins and blood sugar metabolism.

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CONFLICT OF INTERST

Note: This investigation was entirely funded by the lead investigator. The author applied for a patent on the extragonadal use of hCG.

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434 **TABLES:**

435 **Table 1.** Adverse events in brain histopathology per group/sex

Brain Histopathology	Group/sex
Vascular congestion in meninges and parenchyma.	Group 4: 1 female Group 6: 1 female
Vascular congestion and erythrocyte extravasation in meninges.	Group 5: 1 female
Focal points of RBC extravasation in parenchyma	Group 0: 1 female
Marked vascular congestion in meninges	Group 5: 1 female Group 6: 1 female

436

437 **Table 2.** Adverse events in testicular histopathology per group.

Testicular histopathology.	**Groups**						
	0	1	2	3	4	5	6
Mild autolysis	2	2	3	2			
Moderate autolysis	1		1	3	3	3	1

438

439 **Table 3.** Adverse events in ovaries histopathology per group.

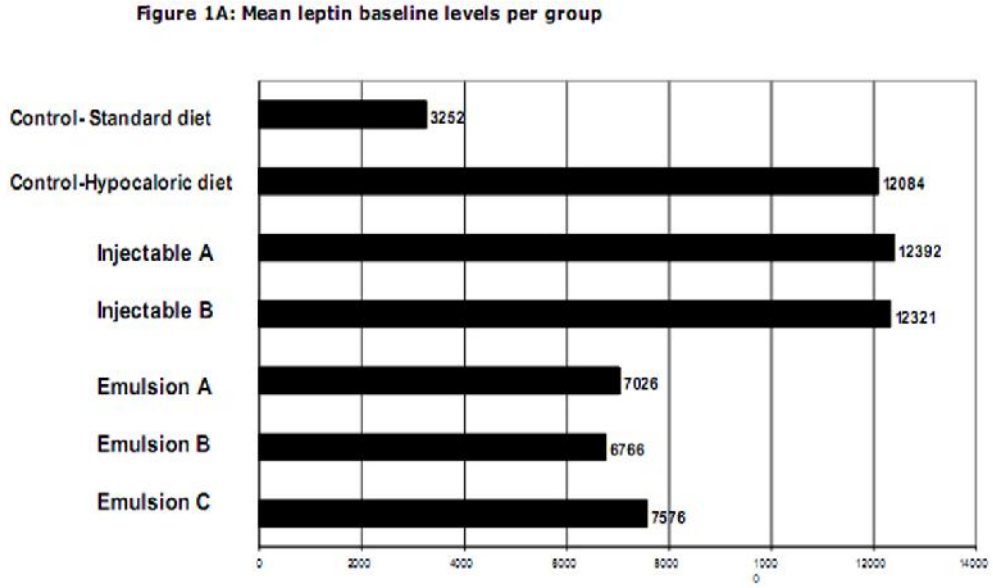
Ovary histopathology.	**Groups**						
	0	1	2	3	4	5	6
CL (Corpus Luteum)							1
Yellowish-brown pigmento focal points.			1				
Follicles in different maturation stages	2	1	3	2	3	5	2
Corpus Luteum in different maturation stages	1						
Interstitial cell hyperplasia.	2						
Interstitial cells hyperplasia and hypertrophy.	1	1	3	2	3	3	3
Interstitial cells mild hyperplasia.		2					
Luteomas	1	1	3	2	3	3	3
Pigment in CL				1	1		
Cysts			2	2	2		

440

441 **FIGURES**

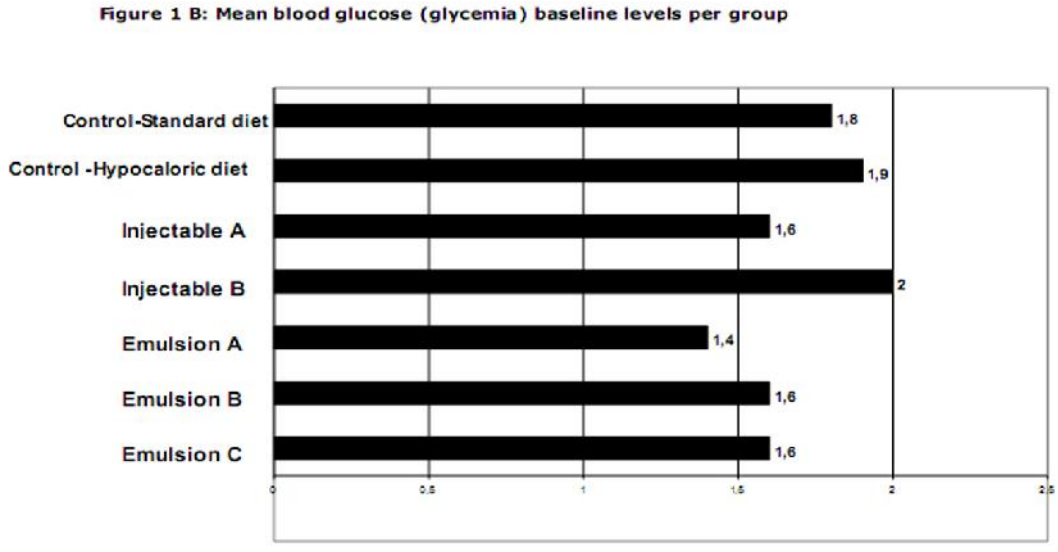
442 **Figures 1(A—D).** Mean baseline determinations per group

443 **Figure 1A.** Mean leptin baseline levels per group



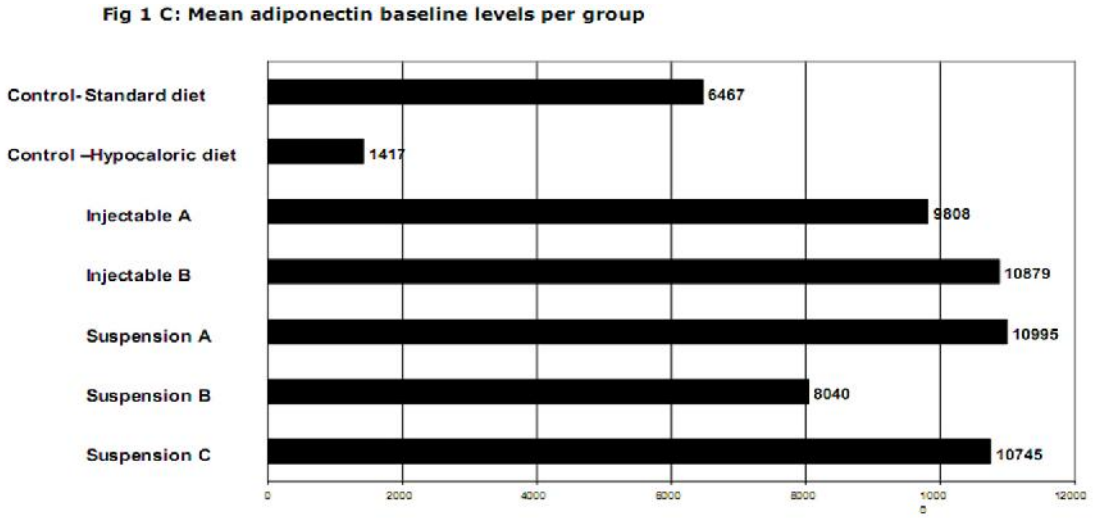
444

445 **Figure 1B**. Mean blood glucose (glycemia) baseline levels per group



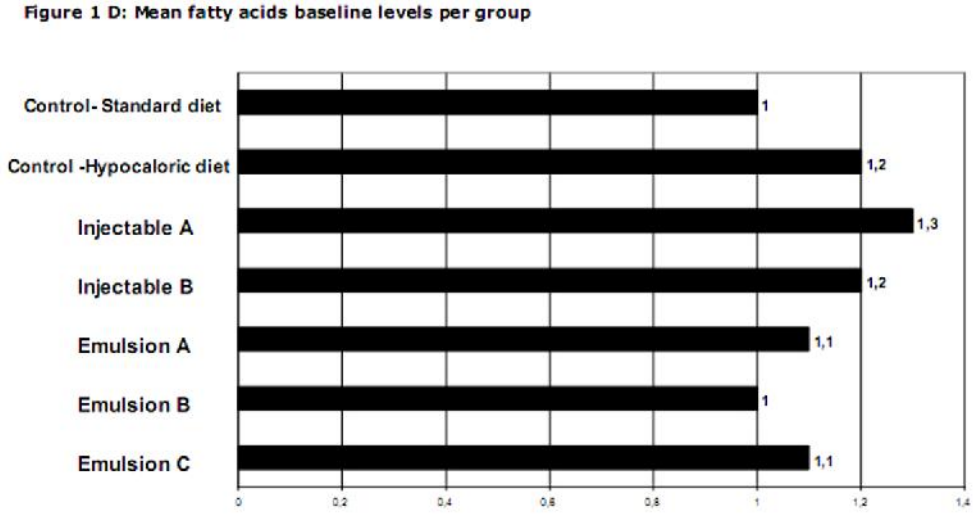
446

447 **Figure 1C.** Mean adiponectin baseline levels per group



448

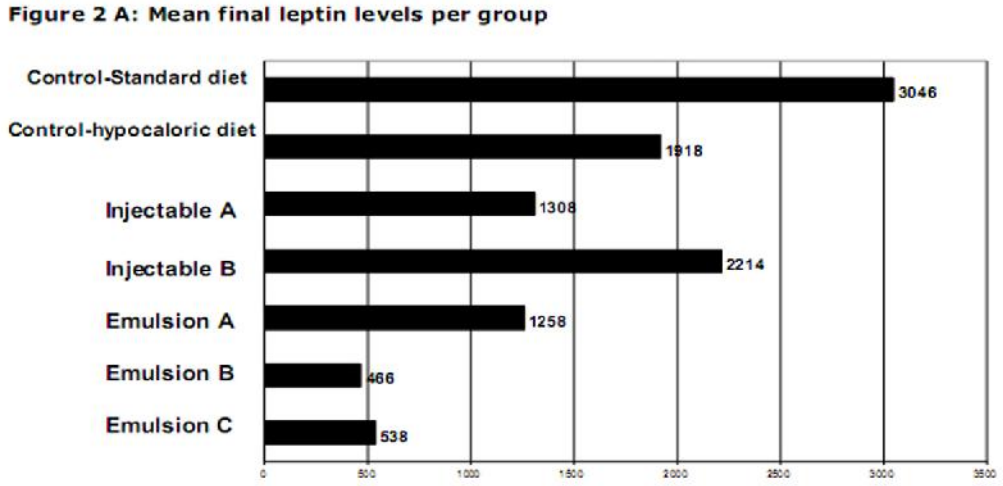
449 **Figure 1D**. Mean fatty acids baseline levels per group



450

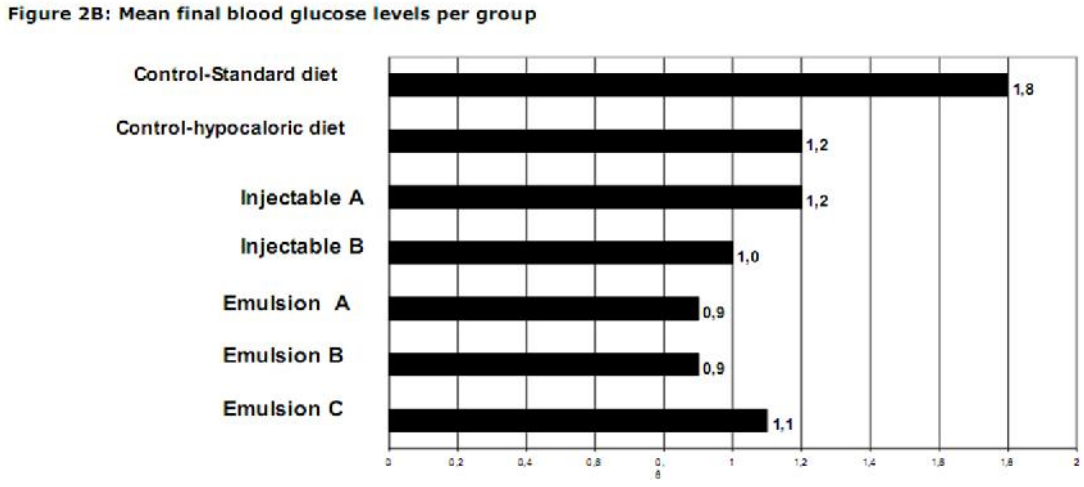
451 **Figures 2(A—D).** Mean final determinations per group

452 **Figure 2A.** Mean leptin final levels per group



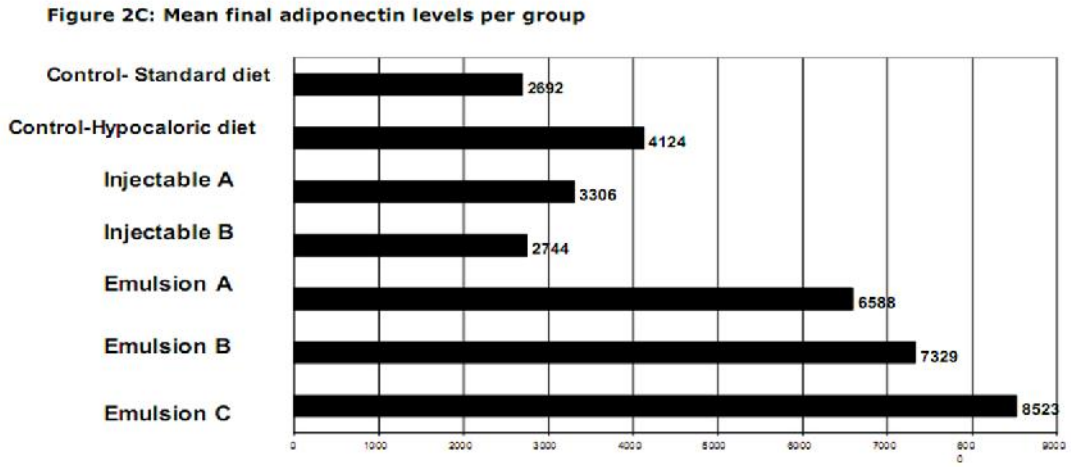
453

454 **Figure 2B.** Mean blood glucose (glycemia) final levels per group



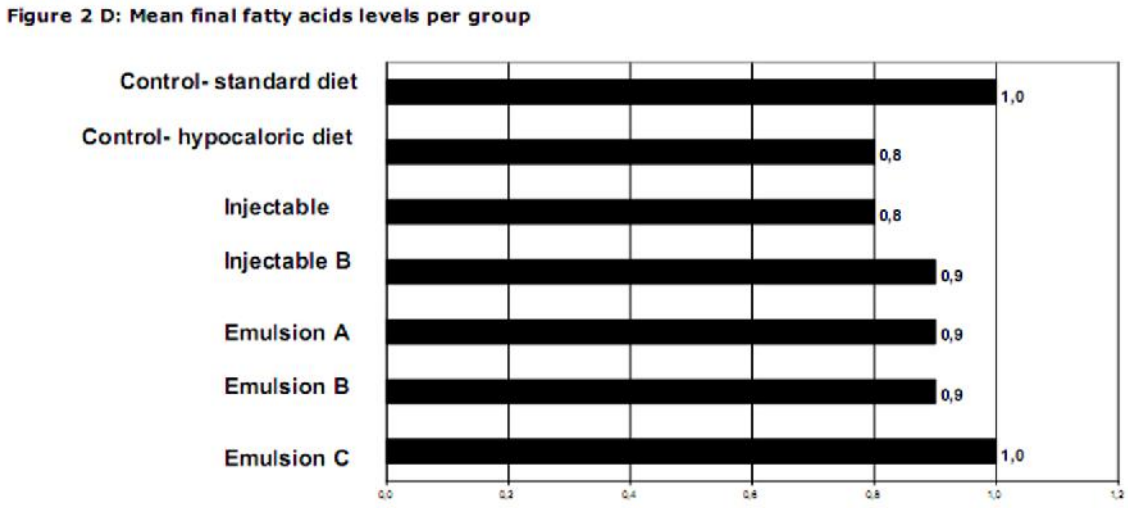
455

456 **Figure 2C.** Mean adiponectin final levels per group



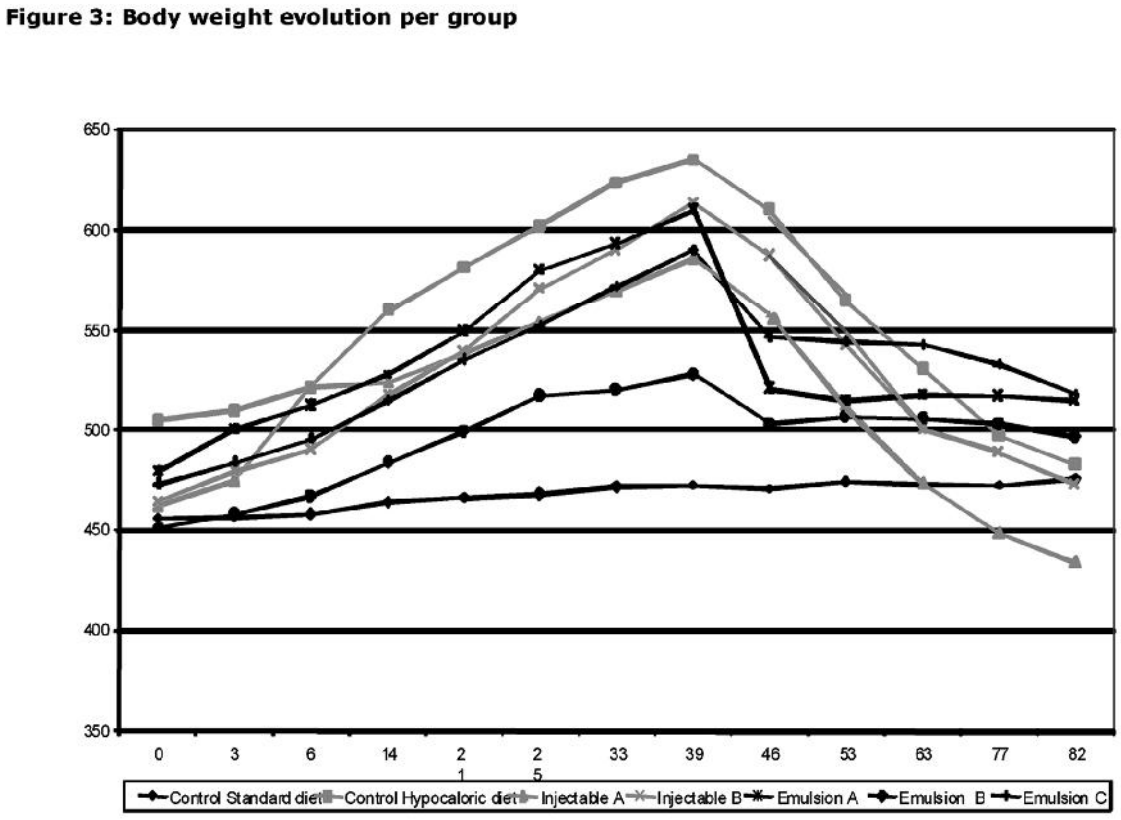
457

458 **Figure 2D.** Mean fatty acids final levels per group



459

460 **Figure 3.** Body weight modifications per group



461

Figure 1A: Mean leptin baseline levels per group

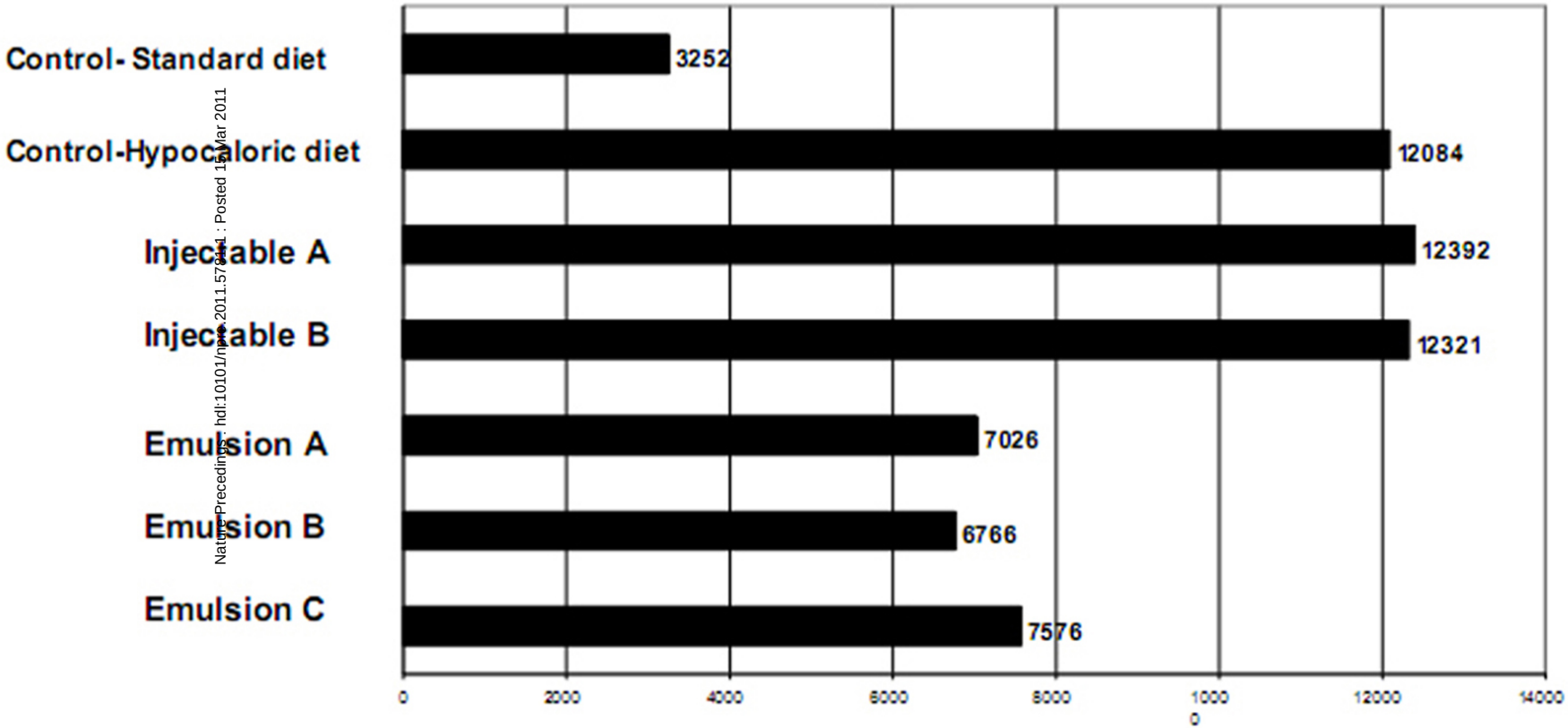


Figure 1 B: Mean blood glucose (glycemia) baseline levels per group

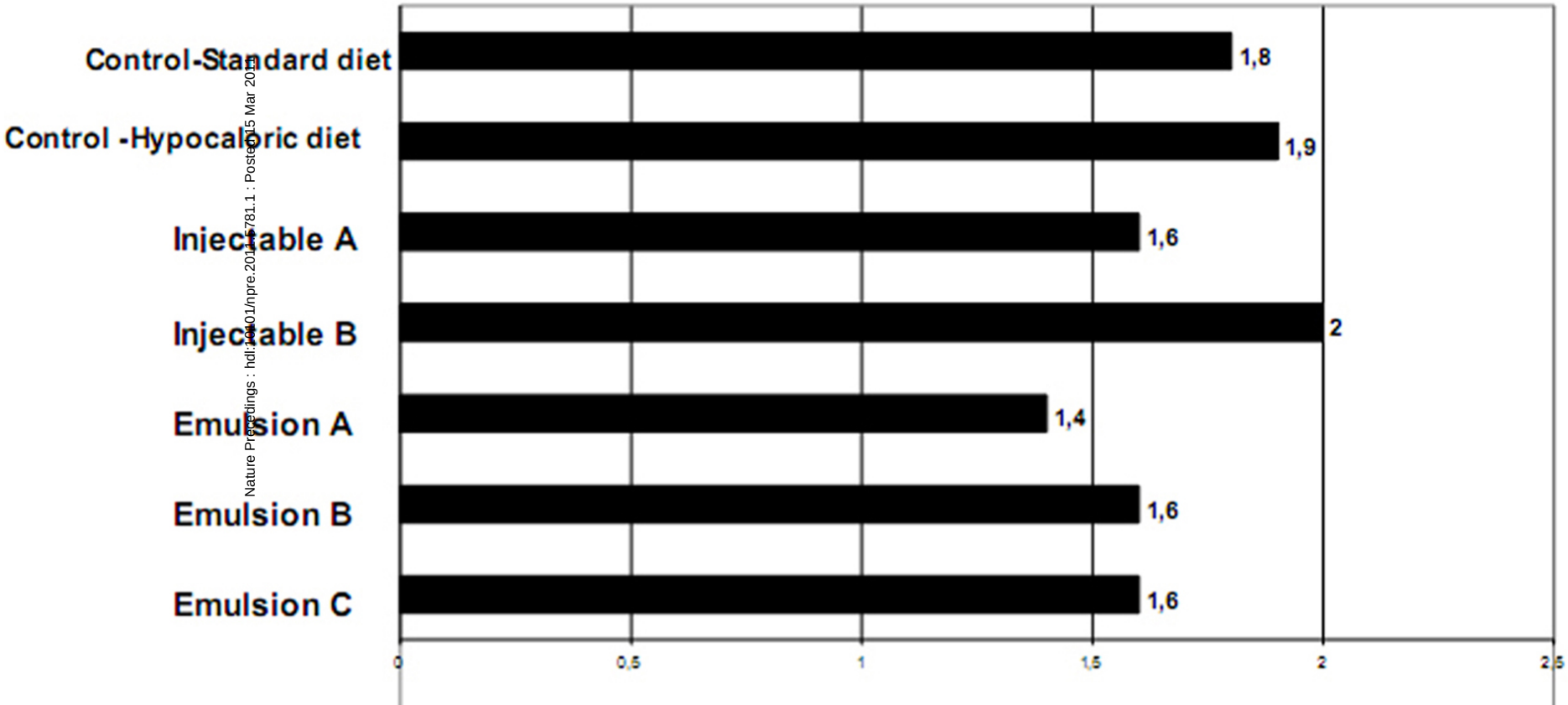


Fig 1 C: Mean adiponectin baseline levels per group

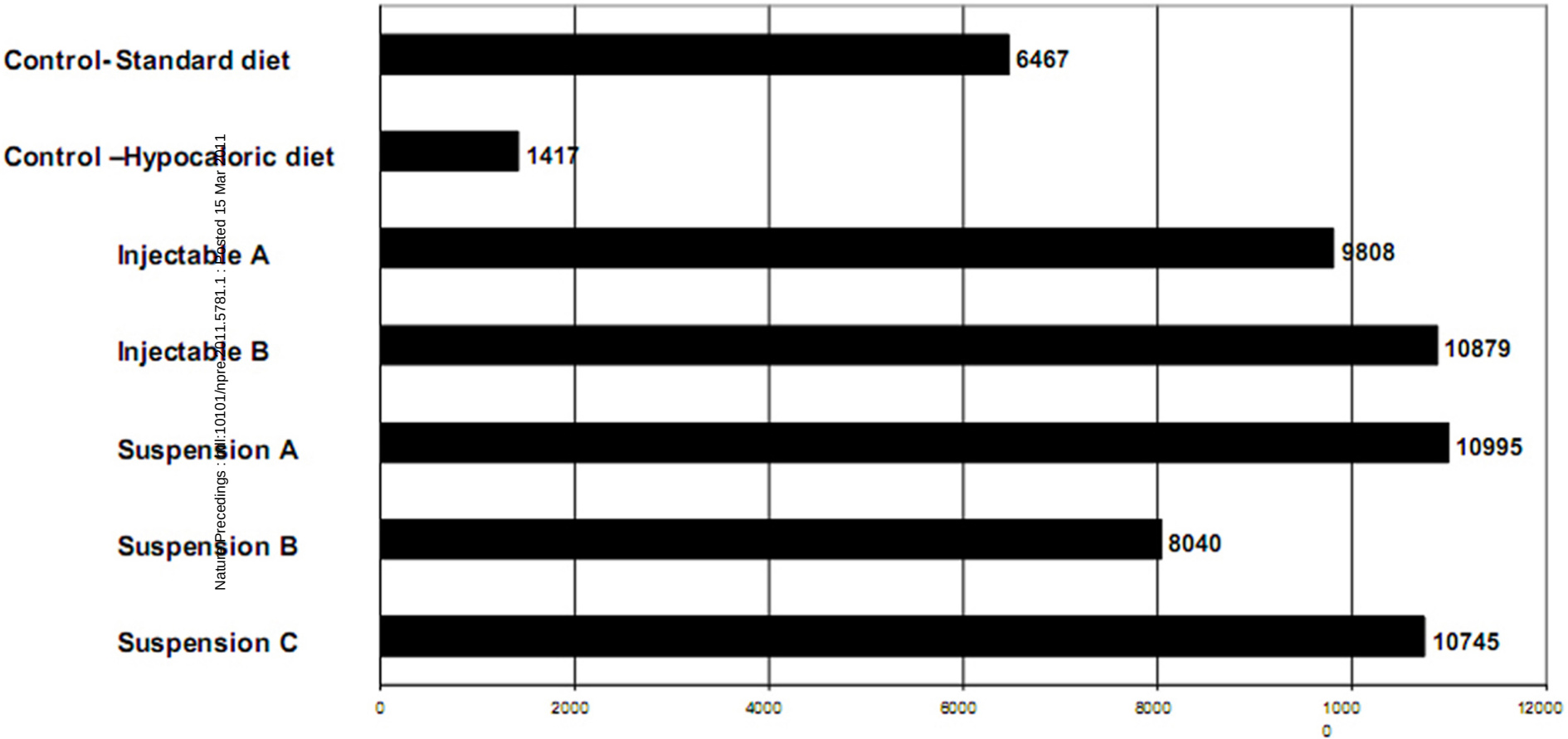


Figure 1 D: Mean fatty acids baseline levels per group

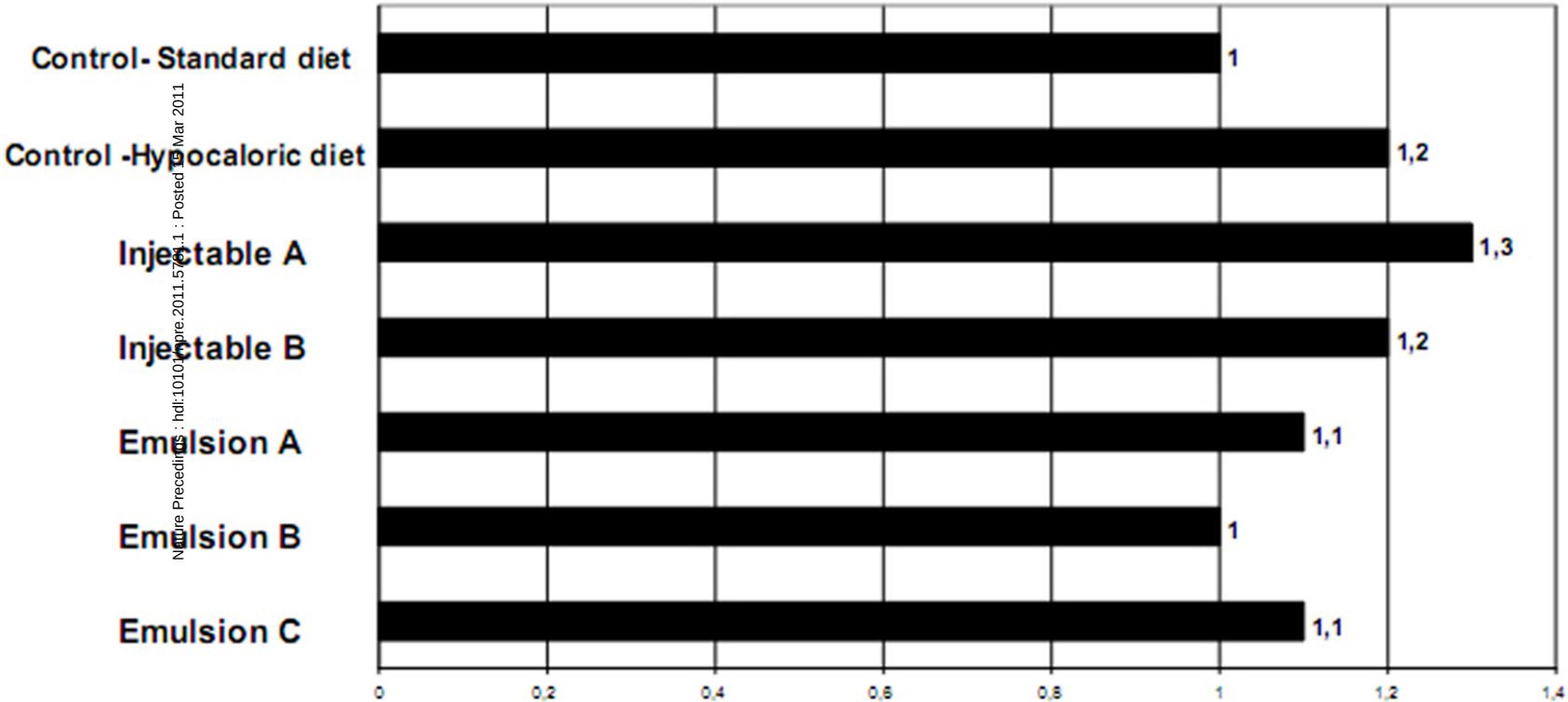


Figure 2 A: Mean final leptin levels per group

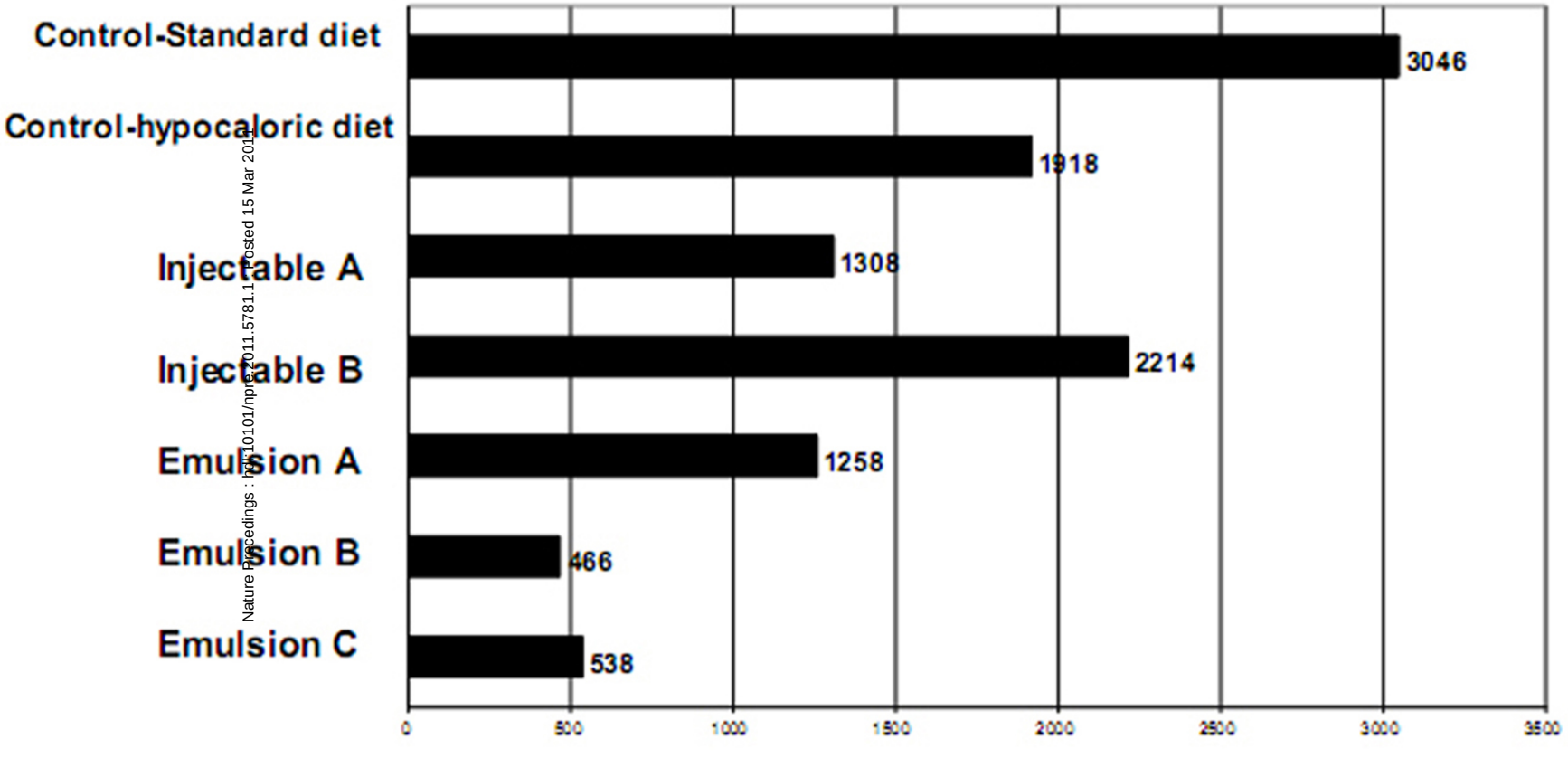


Figure 2B: Mean final blood glucose levels per group

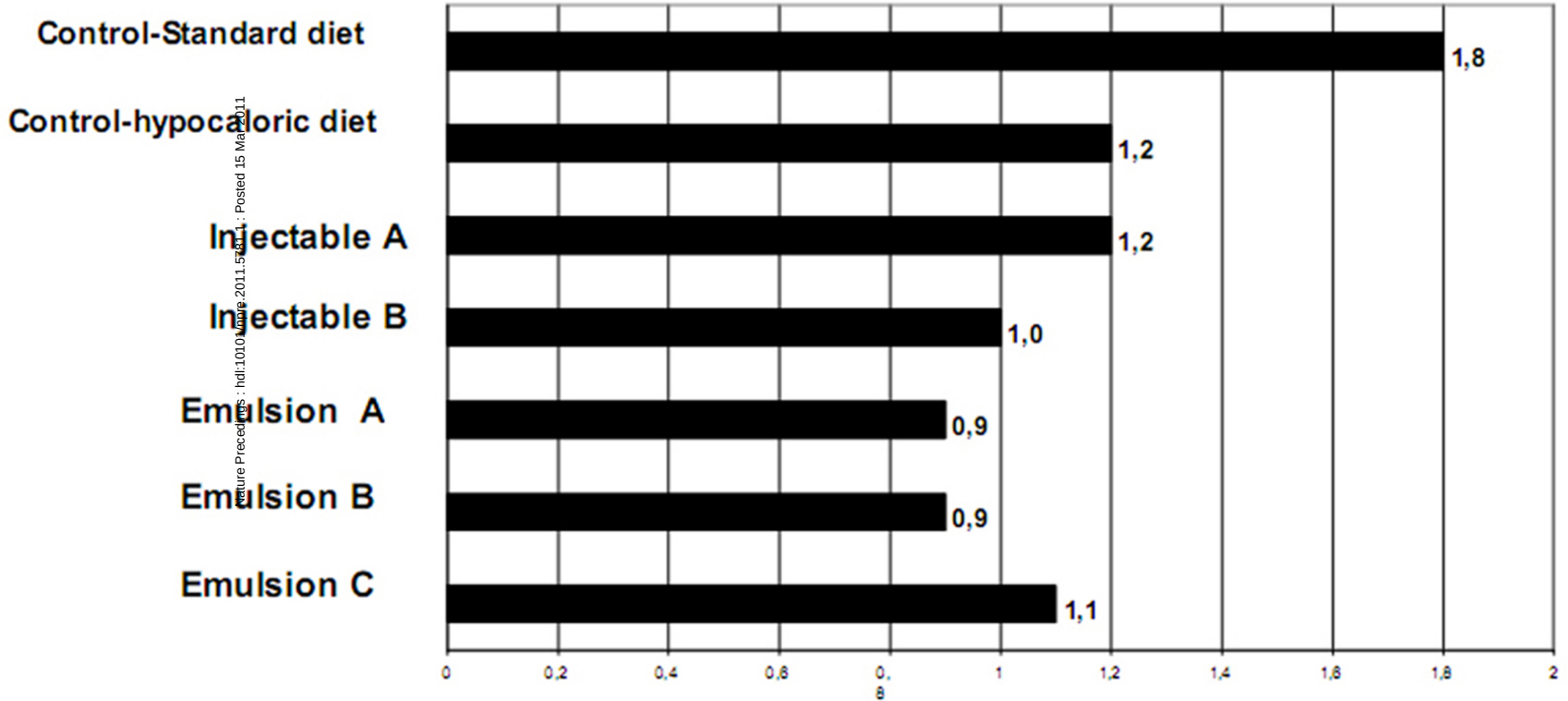


Figure 2C: Mean final adiponectin levels per group

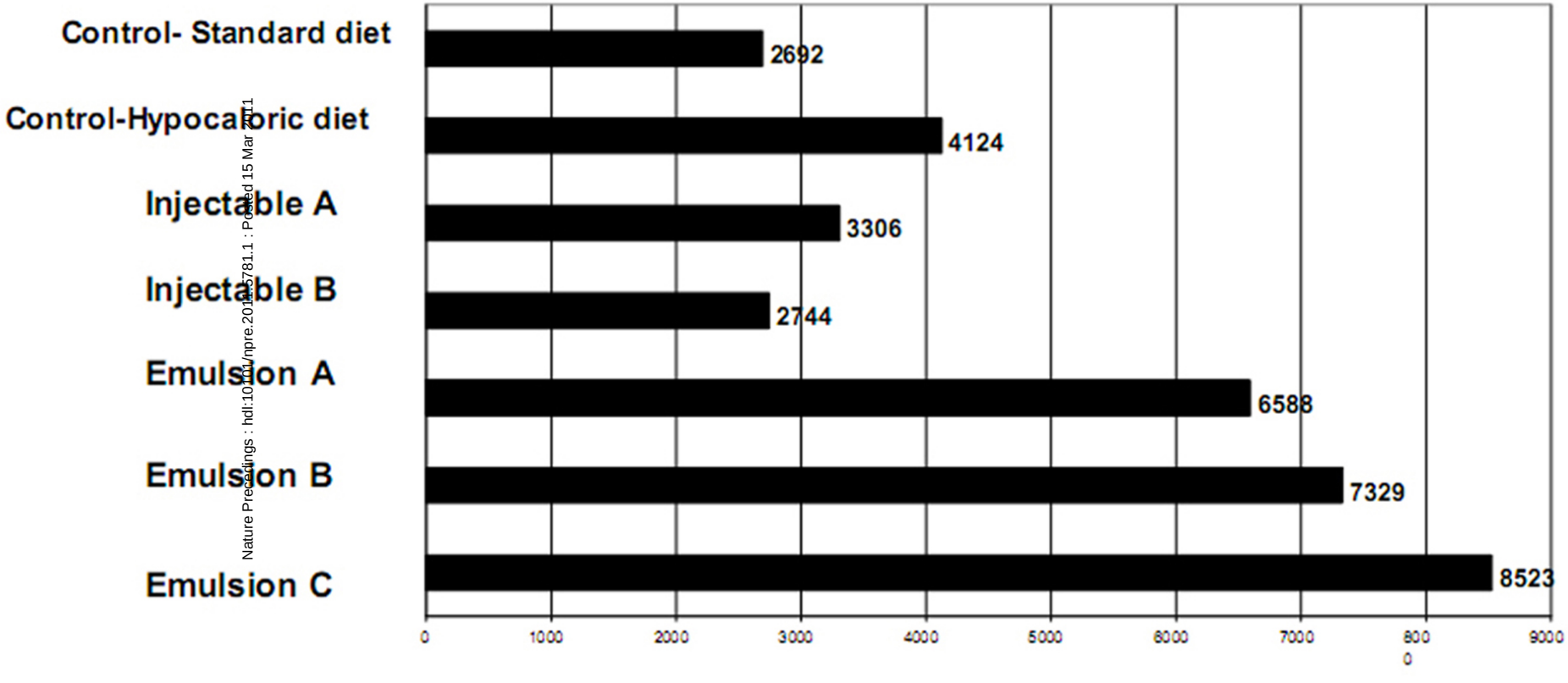


Figure 2 D: Mean final fatty acids levels per group