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ORIGINAL ARTICLE

Diet-induced obesity and associated disorders are prevented by natural bioactive type 1 fish collagen peptides (Naticol®) treatment

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Abstract

To fight against metabolic disorders such as insulin resistance, new alimentary behaviors are developed. For instance, hyperproteined, gluten-free, or collagen-enriched diets could be preconized in order to reduce the consequences of obesity. In this aim, this study evaluates the potential effects of warm sea fish collagen peptides (Naticol®) on representative metabolic and inflammatory parameters. For that, male C57Bl6/J mice fed with either a chow- (CD) or high-fat diet (HFD) were submitted or not to specific collagen peptides in drinking water (4 g/kg bw/d) for 20 weeks. Weight, body composition, glucose tolerance, and insulin sensitivity were followed up. Effects of fish collagen peptides on various blood parameters reflecting the metabolism status were also measured (free fatty acids, triglycerides, cholesterol, hormones) together with adipocyte inflammation. Results showed that HFD-fed mice supplemented by fish collagen peptides exhibited a significant lower increase in body weight as soon as the twelfth week of treatment whereas no effect of the peptide was observed in CD fed mice. In line with this result, a weaker increase in fat mass in HFD-fed mice supplemented with Naticol® at both 9 and 18 weeks of treatment was also observed. In spite of this resistance to obesity promoted by fish collagen peptides treatment, no difference in glucose tolerance was found between groups whereas mice treated with Naticol® exhibited a lower basal glycemia. Also, even if no effect of the treatment on adipocyte lipolysis was found, a decrease of inflammatory cytokines was retrieved in collagen-supplemented group arguing for a potential better insulin sensitivity. Altogether, these results need to be completed but are the first describing a benefic role of warm sea fish collagen peptides in a context of metabolic disease paving the route for a potential utilization in human obesity-associated disorders.

Keywords Collagen · Glycemia · Adipose tissue · Obesity · Low-grade inflammation · Adipokine

Introduction

Obesity is defined as an abnormal or excessive fat accumulation that may impair health. This excess of adipose tissue results in a chronic low-grade inflammatory state (macrophage infiltration, TNFα, IL-6, …) leading to insulin pathway alteration. Consequently, obesity is strongly associated with insulin resistance, which, when coupled with relative insulin deficiency, leads to the development of type 2 diabetes. As the incidence of diabetes is increasing worldwide and currently affects more than 194 million individuals [20], it will become one of the major public health concerns in the next decades. Among the different types of diabetes, type 2 diabetes mellitus (T2DM) is highly prevalent, accounting for about 90% of the total diabetic patients [10]. In T2DM, patients often face the problem of lack of insulin sensitivity. Both insulin resistance and β-cell dysfunction are important parameters of T2DM [1]. During the pathogenic process of T2DM, many factors contribute to the development of insulin resistance and β-cell dysfunction including genetic, environmental, and individual behavioral factors, such as overweight, sedentarily, hypertension, and stress.

T2DM is believed to be associated with obesity, considered to be caused by unhealthy eating habits and lifestyle patterns [2, 16]. A large number of studies have reported the strong relationship between obesity/overweight and the onset of type 2 diabetes [7, 18]. Conversely, they have shown that increased physical exercise, weight loss, or adoption of a certain diet may reduce the incidence of T2DM [4, 13].

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In this context, different new alimentation behaviors are considered such as collagen peptides from warm sea fishes. Collagen is the major structural protein present in skins and bones of all animals where it accounts for 30% of the total protein content [8]. Type I and III collagens are synthesized from precursor called pro-collagen which is derived from dermal fibroblasts. Collagen synthesis is increased by transforming growth factor-β (TGF-β), a cytokine that promotes collagen production, and activator protein-1 (AP-1), a transcription factor that promotes collagen breakdown by up regulating matrix metalloproteinases. Collagen is mainly sought for the production of gelatin, a high value functional protein due to its unique gel-forming capacity.

In 2009, Veldhorst et al. [15] reported that gelatin alone or gelatin added with tryptophan and alpha-lactalbumine were 40% more satiating than other proteins (casein, soy, whey, or whey-GMP) and induced a related 20% reduction of subsequent energy intake.

Other studies showed that treatment with dietary cod proteins improved the insulin sensitivity in insulin-resistant individuals and reduced insulin-resistance-related metabolic disorders, contributing to the prevention of T2DM [5, 12]. Treatment with oligopeptides from marine salmon (*Oncorhynchus keta*) skin demonstrated inhibited inflammation by reducing the production of proinflammatory cytokines in mice [22]. More recently, a study showed that oral L-arginine acted as a glucagon-like peptide 1 (GLP-1) to increase postprandial insulin secretion and improve glucose tolerance. An increasing number of studies have been performed to investigate the metabolic effects of dietary proteins on insulin /glucose homeostasis and satiety but until now, there are no conclusions demonstrating the effects of collagen peptides from warm sea fishes on body composition and metabolic parameters (glycemia, insulinemia, cholesterolemia, triglycerides, and free fatty acids), in obese non-insulin dependent type 2 diabetes subjects.

The present article aimed to evaluate the potential effects of warm sea fish collagen peptides on representative metabolic parameters to prompt the development of innovative compounds for the prevention and delay of diabetes and other metabolic-associated disorders.

Materials and methods

Animals

Animals were handled with the principles and guidelines established by the National Institute of Medical Research. Male C57Bl6/j mice were obtained from Charles River Laboratory (L'Arbresle, France). Mice were housed conventionally in a constant temperature (20–22 °C) and humidity (50–60%) animal room, with a 12/12 h light/dark cycle and free access to food and water. In the supplemented diet, Naticol®-2 kDa® (4 g/kg/day) were added to the drinking water (in water, Naticol® have no specific color, taste, or smell). Water intake was first evaluated to use the appropriate dilution of Naticol®. For that purpose, food/water intake was measured 2 days and 6 weeks after the beginning of the treatment. Mice were housed individually on a 12-cm-diameter grid of metabolic cages (BioSeb, Spain). They were given free access to food and water, and the food/water was weighed every 12 h during 48 h, after 24 h of acclimatization. When fed a high fat diet (HFD), energy contents of the specific diets were (percent kilocalories): 20% protein, 70% carbohydrate, 15% fat for CD and 20% protein, 20% carbohydrate, and 60% fat for HFD. The main source of fat in HFD was lard (20 g per 100 g of food). During the treatment, mice were followed every week with measurement of body weight until 20 weeks of treatment.

Body mass composition

To determine fat and lean mass, mice were placed in a clear plastic holder without anesthesia or sedation and inserted into the EchoMRI-3-in-1 system from Echo medical systems (Houston, TX, USA). Total body fat and lean mass as well as water content were measured at 9 and 18 weeks of treatment and whole body fat gain was calculated.

Product

The products used in this study were fish collagen peptides derived from a special enzymatic hydrolysis of fish type I (mainly) and III collagen. The products were provided by WEISHARDT (Graulhet, France), commercially available under the name Naticol®. Naticol®-2 kDa represents a blend of specific fish collagen peptides obtained from warm sea fish skins with a mean molecular weight (Mw) evaluated by standardized GME (Gelatine Manufacturers of Europe, leading association of Europe's foremost gelatine manufacturers supporting the products gelatine and collagen peptides and also informing, educating, and communicating with customers and authorities) method as equal to 2 kDa. Amino acids composition of Naticol is presented in Table 1. Control group in CD and HFD diet received tap water.

Oral glucose tolerance test

OGTT was performed at week 9 and 18 of the protocol. After 6 h fast, mice received glucose (3 g/kg body weight) by gavage through a gastric tube (outer diameter 1.2 mm). Glycemia was monitored from the tail vein 0, 15, 30, 45, 60, 90, and 120 min after glucose administration, using a glucometer (Accu-check, Roche Diagnostic, Grenoble, France). The area under the curve was calculated.

Table 1 Amino acid composition of Naticol® (Fish collagen peptides, Weishardt). Values are expressed in percentage of each amino acid reported to all amino acids. Tryptophan has been retrieved as traces

	% of amino acid
Glycine	20.9
Proline	12.6
Glutamic acid	11.6
Hydroxyproline	10.5
Arginine	8.9
Alanine	8.3
Aspartic acid	5.1
Lysine	3.5
Serine	3.5
Threonine	2.7
Leucine	2.6
Phenylalanine	2.3
Valine	2.0
Isoleucine	1.5
Hydroxylysine	1.5
Histidine	1.3
Methionine	0.8
Tyrosine	0.4
Cysteine + cystine	0.03

Adipocyte preparation and lipolysis

Isolated adipocytes were obtained after mincing adipose tissue in 5 mL of Dulbecco's modified Eagle's medium (DMEM, Gibco, Invitrogen, Paisley, UK) supplemented with 1 mg/mL collagenase and 1% albumin (BSA) for 30 min at 37 °C under shaking. Adipose tissue parts were digested by collagenase (type II, Sigma-Aldrich Co, St Louis, MO) in order to get isolated adipocytes. Digestion was followed by filtration through a 150-μm screen and the floating adipocytes were separated from the medium containing the stroma vascular fraction and washed twice in DMEM.

Lipolysis was realized in KRHB (Krebs Ringer Hepes buffer with 2% BSA). One hundred microliters of adipocytes (1/10 dilution) were incubated in 1 mL of KRHB for 1 h at 37 °C under gentle shaking in the presence of different concentrations of a lipolytic agent: isoprenaline (a β-adrenergic agonist). The reaction was stopped once the tubes were in ice. Glycerol released in the medium was measured on 30 μL aliquot using the Glycerol Free Reagent kit (Sigma) while non esterified fatty acids (NEFA) were measured on 15 μL of medium by the WAKO NEFA kit (WAKO chemicals).

Plasma parameters

After 12 h overnight fast, Naticol®-treated or untreated mice were killed by cervical dislocation and blood was collected in EDTA-wetted syringes from the descending vena cava. Blood glucose levels were immediately measured with a gluco-meter (Accu-check Actine, Roche) and plasma was separated by centrifugation at 5000 x g for 10 min. Plasma samples were immediately frozen for further analyses. Plasma-free fatty acids (FFA), cholesterol, and triglycerides (TG) were determined in the fed state at the end of the treatment in 5 μL of plasma collected from the tail blood, by an enzymatic colorimetric technique with the Wako NEFA kit (Wako Chemicals). Plasma insulin and IGF1 levels were measured using an ELISA Kit (Mercodia, Uppsala, Sweden).

Gene expression study

Immediately after euthanasia, adipose tissue was taken and frozen in liquid nitrogen, and total RNAs were isolated using the GeneJET RNA Purification kit (Fermentas). Total RNAs (500 ng) were reverse transcribed using Superscript II reverse transcriptase (Invitrogen, UK) in the presence of a random hexamer. The same reaction was performed without Superscript II to estimate DNA contamination. Real-time PCR was performed as previously described (Boucher et al. 2005). Analysis of HPRT expression was performed to normalize gene expression.

Statistical analysis

Data are presented as means ± SEM. Analysis of differences between groups was performed with Student's t test, and $p < 0.05$ was considered to be significant.

Results

Body weight and composition

Whatever the regimen used (chow diet (CD) or high fat diet (HFD)), the Naticol®-supplemented diet did not alter food or water intake (Fig. 1c). Moreover, in the CD fed groups, the body weight did not vary between the groups during the 20 weeks of the experiment. In the HFD groups, Naticol®-supplemented diet promoted a significantly lower increase in body weight as soon as the twelfth week of treatment (Fig. 1a, b). As shown in Fig. 2, this decrease of body weight is due to a lower increase in fat mass in HF fed mice supplemented with Naticol®. Surprisingly, body composition was significantly altered in Naticol®-supplemented group in CD at week 18 although no variation in total body weight was observed. Whatever the group studied, no significant variation of lean mass and water content was found (Fig. 2b, c).

Plasma metabolic parameters

Regarding the carbohydrate-related parameters, we observed a lower rise in basal plasma glucose levels in

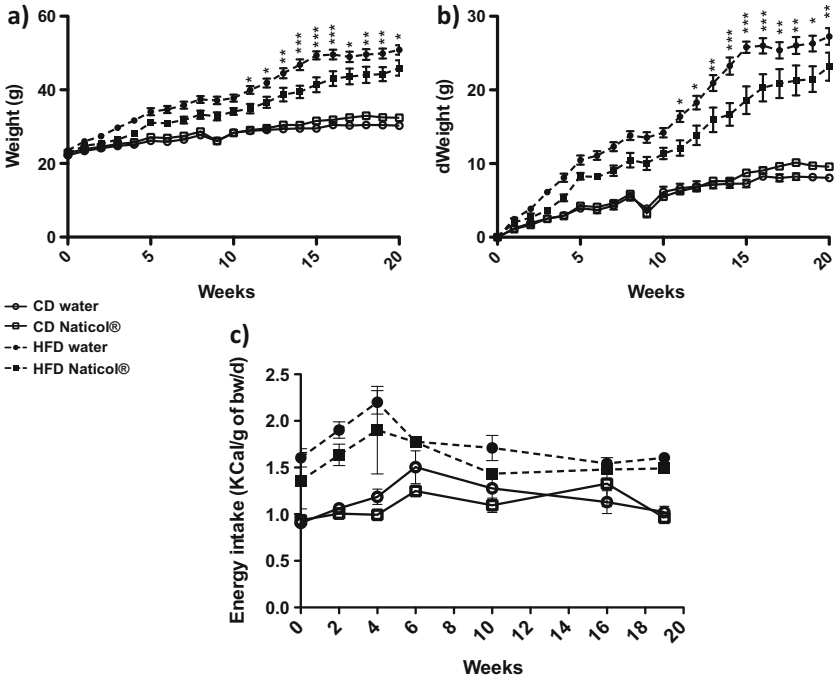

Fig. 1 **a** Body weight time course and **b** variation in body weight time courses of either chow- (empty symbols) and high fat- (full symbols) fed (CD and HFD respectively) mice supplemented with Naticol® (squares) or not (circles). **c** Represents the food intake in CD and HFD mice supplemented or not by Naticol®. Data are presented as mean ± SEM ($n = 6$)

HFD groups when they were Naticol®-supplemented (Fig. 3a). However, no modification of glucose tolerance was obtained after glucose load in the different group of mice (Fig. 4a, b). Other blood metabolic-related parameters have been measured (Fig. 3) and showed that HFD-fed mice supplemented with collagen exhibited higher levels of triglycerides with lower amount of plasma cholesterol (Fig. 3e, f). Finally, a slight but not significant decrease in plasma insulin was found in the same groups of mice (Fig. 3b).

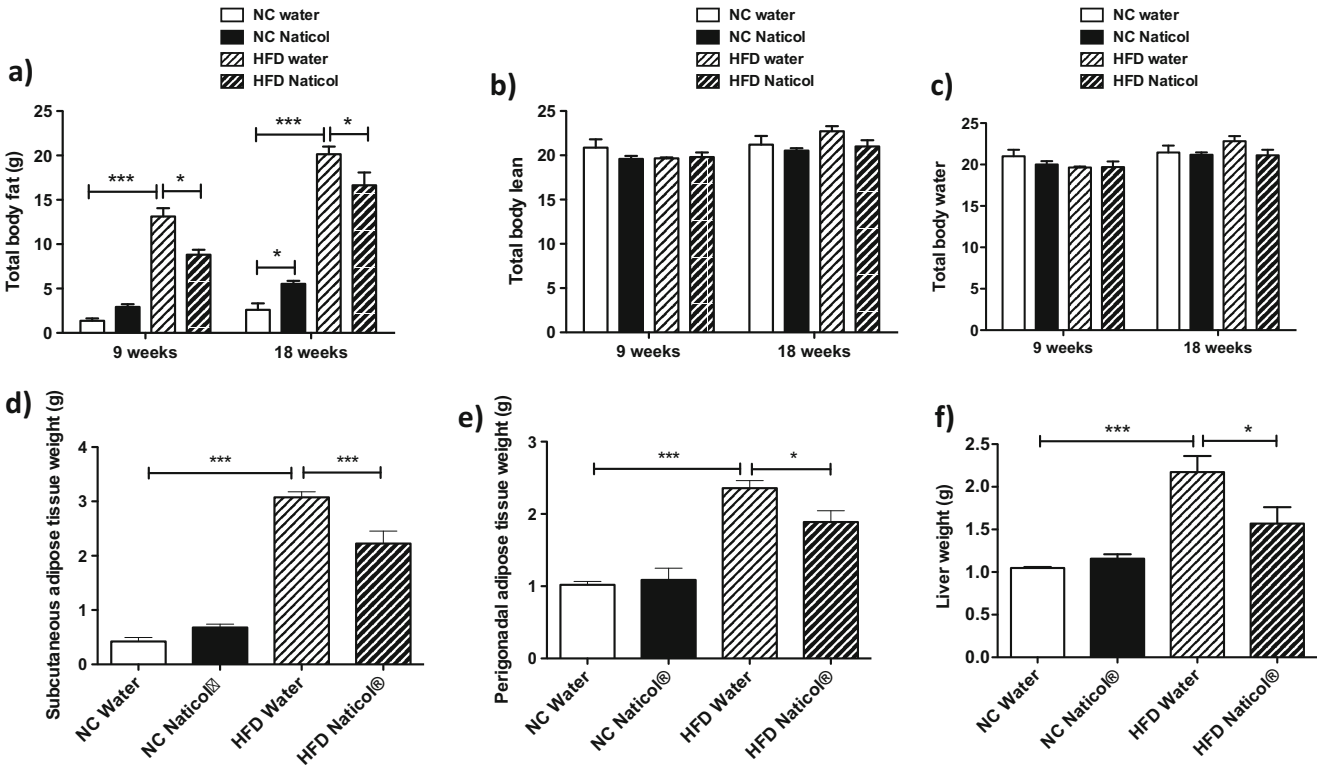

Fig. 2 Assessment of body composition in mice (hatched bar when high fat fed) supplemented 9 and 18 weeks with Naticol® (black bars) or not (white bars). Fat stores **a**, lean mass **b**, and total water **c** are presented as mean ± SEM ($n = 6$)

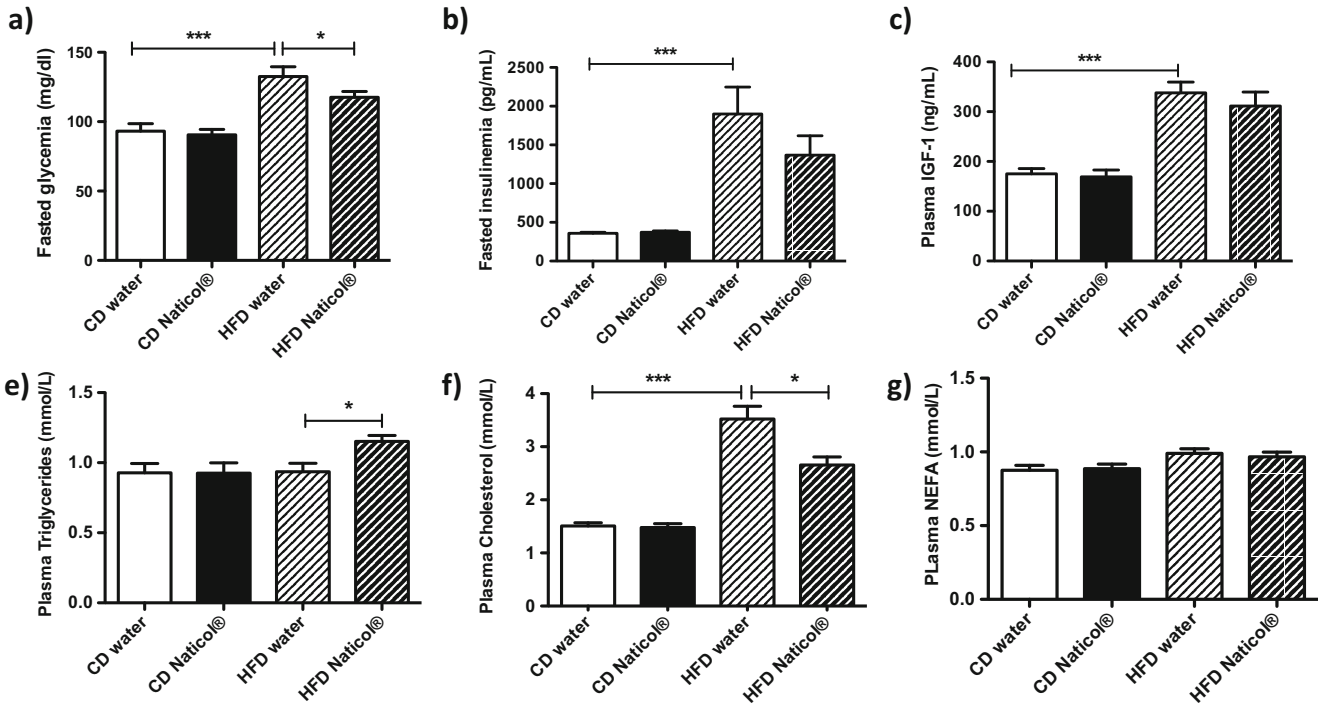

Fig. 3 Blood carbohydrates related and lipids in mice (hatched bar when high fat fed) supplemented 20 weeks with Naticol® (black bars) or not (white bars). Plasma glucose **a**, insulin **b**, IGF-1 **c**, triglycerides **d**, cholesterol **e**, and NEFA **f** are presented as mean ± SEM ($n = 6$)

Adipose tissue function and inflammatory profile

To better understand the mechanisms by which Naticol® induced weight gain reduction associated to an enhanced control of plasma glycemia, we measured functional capacities and inflammatory profile in isolated adipocytes (Fig. 5). Surprisingly, in the chow diet group, the lipolytic activity was induced in Naticol®-supplemented mice (Fig. 5a). When fed a high fat diet, no modification of lipolytic activity was observed in adipocytes from both control and Naticol®-supplemented.

In adipocytes collected from Naticol®-supplemented mice, the inflammation-related gene IL6 was decreased in both CD and HFD groups (Fig. 5b). IL-1β was decrease by Naticol® treatment only in HFD group (Fig. 5c) and TNF-α did not significantly vary (Fig. 5d) while the antidiabetic adipokine apelin was increased (Fig. 5e). No significant variation in the expression of the adipocyte-related gene studied such as leptin, fatty acid binding protein-4 (FABP4), and hormone sensitive lipase (HSL) were observed data not shown.

Discussion

The present data showed the beneficial effects of fish collagen peptides, so-called Naticol®, on body weight and composition under HFD. Fat mass was decreased while lean mass and water content did not show variation in HF-fed mice supplemented with Naticol® after 9 weeks of treatment. These results were not demonstrated in the CD group treated by fish collagen peptides.

The lower gain of weight and fat mass may be linked to a delay of obesity and consequently an improvement of metabolic factors such as glycemia, insulinemia, and cholesterolemia as described by previous studies [4, 13].

It is also well established that obesity is associated with type 2 diabetes, hypertension, and hyperlipidemia. A moderate weight loss has a beneficial effect on the cardiovascular risk factors associated with obesity [17, 21]. Weight reduction (5–10%) is associated with an improvement in fasting glycemia, blood pressure, and plasma lipid profile/cholesterol levels [14]. The UK Prospective Diabetes Study showed a significant correlation between the amount of weight loss during 3 months' dieting after the diagnosis of type 2 diabetes and the decrease in fasting plasma glucose over the same time period [3, 19]. Vidal et al. also reported that modest weight loss could help to prevent the most common condition associated with obesity: type 2 diabetes [17]. Consequently, the lower rise in plasma glucose and the clear absence of increase in plasma cholesterol levels recorded in HF fed mice supplemented with Naticol® may be correlated to the lower weight gain and the lower rise of fat mass. It is also well documented that dietary supplementation with L-arginin improves glycemic control [6]. Collagen is a protein which represents a good source of L-arginin. It may explain the result obtained in HFD for the glycemic control in Naticol®-supplemented groups.

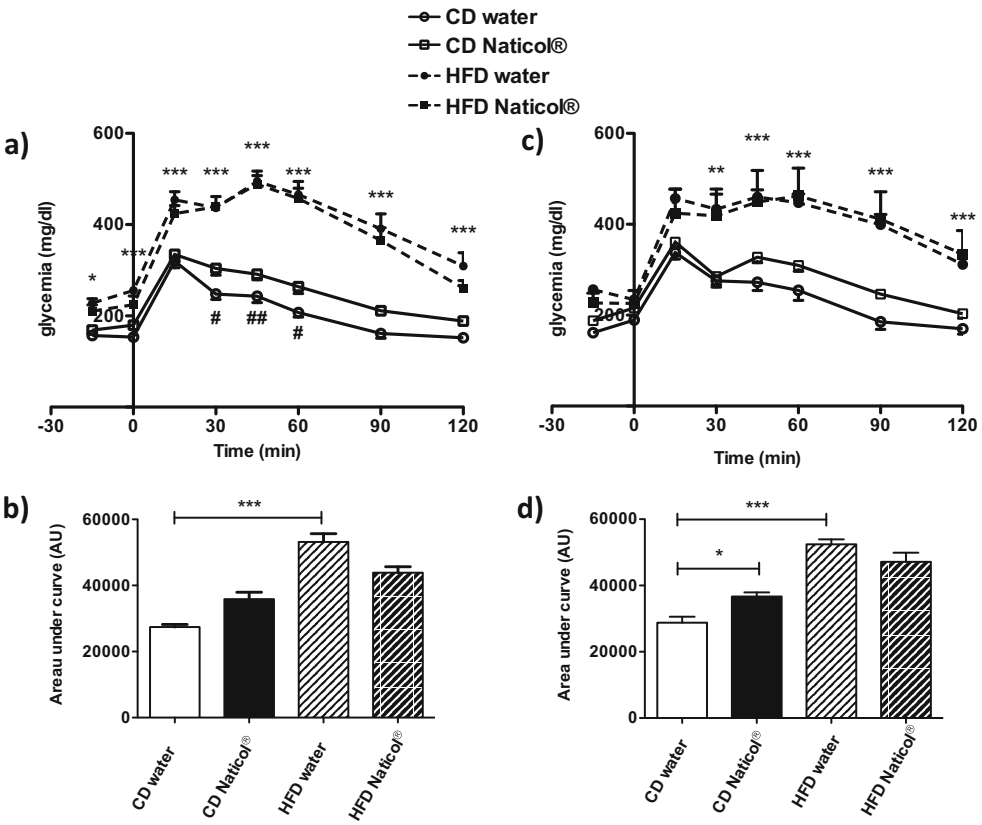

Fig. 4 Oral glucose tolerance test in 6-h-fasted mice performed in mice of either chow- (empty symbols) and high fat-(full symbols) fed (CD and HFD respectively) mice supplemented 9 **a** and 18 **b** weeks with Naticol® (squares) or not (circles). Data are presented as mean ± SEM ($n = 6$)

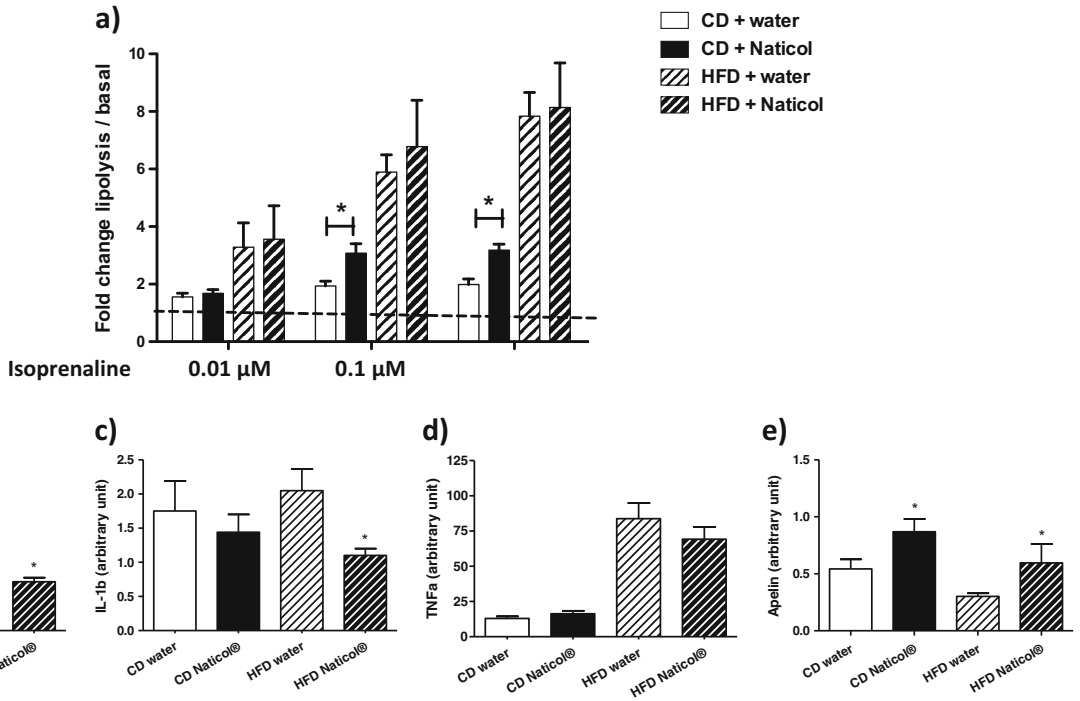

Fig. 5 Functional activity and inflammatory profile in isolated adipocytes. **a** Fold change in glycerol release upon isoprenaline-stimulated lipolysiseither chow mice supplemented with Naticol® or not. Data are presented as mean ± SEM ($n = 6$). **b**, **c**, **d**, **e** Gene expression profile in mice (hatched bar when high fat fed) supplemented 20 weeks with Naticol® (black bars) or not (white bars). Interleukin-6 (IL-6, **b**), Inteleukin-1β (IL-1β, **c**), Tumor necrosis factor-α (TNF-α, **d**), and apelin **e** mRNA quantifications are presented as mean ± SEM ($n = 6$)

Regarding lipid-related parameters, plasma cholesterol was significantly decreased by the treatment under HF conditions. This result suggests that Naticol® may act by reducing cholesterol uptake from alimentation or efflux from liver. Since this effect could be essential to fight against high cholesterolemia-associated pathologies, further experiments are needed to identify the potentials targets of Naticol®. Surprisingly, CD mice chronically submitted to Naticol® exhibited a strong increase of basal adipocyte lipolysis. The explanation may reside in the fact that Naticol®-supplemented mice presented a lower level of glycemia after glucose load and consequently needed to mobilize lipid from adipose tissue.

Results also showed that TNF-α did not significantly vary even if it tended to decrease in Naticol®-supplemented groups, in HFD. However, IL-6 and IL-1β are reduced in adipose tissue of Naticol® treated mice concomitantly with an increase of the insulin-sensitizer cytokine apelin. These results suggested that Naticol® could directly or indirectly target inflammatory processes to improve metabolism of adipose tissues in non-obese or obese mice. Indeed, IL-1β is a multifunctional pro-inflammatory cytokine produced by numerous innate immune cells including monocytes and macrophages [11]. Increased circulating IL-1β concentrations have been associated with greater risk of developing type 2 diabetes [9]. Consequently, the decrease of IL-1β in adipose tissue of Naticol®-supplemented groups could be correlated with a reduced risk of type 2 diabetes. IL-6 decrease may also suggest a lower state of adiposity and a mechanism to increase insulin sensitivity during the obese insulin-resistant state. Finally, adipose tissue apelin overexpression during Naticol®-supplemented diet indicated that this peptide could be considered as a potential trigger of Naticol® effects at least in adipose.

Even if the targets and the mechanisms used by Naticol® need to be investigated in the future, this study showed the potential effects of warm sea fish collagen peptides, on representative metabolic parameters to prompt the development of innovative natural collagen peptides for the prevention and delay of diabetes and other metabolic-associated disorders.

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