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BRIEF COMMUNICATION

Clinical Research

Adipose tissue contribution to plasma fibroblast growth factor 21 and fibroblast activation protein in obesity

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Abstract

Fibroblast growth factor 21 (FGF21) is an important regulator of energy metabolism. FGF21 is inactivated by fibroblast activation protein (FAP). We investigated whether FGF21 and/or FAP are secreted from human white adipose tissue of individuals with obesity by measuring total FGF21, active FGF21, and FAP concentrations in arterialized blood and venous blood draining the subcutaneous abdominal adipose tissue (scAT). Measurements were performed under fasting conditions and after a high fat meal before and after diet-induced weight loss in 16 adults with BMI 27–35 kg/m^2. FGF21 was not released from scAT, neither before nor after weight loss in agreement with an undetectable gene expression of FGF21 in this tissue. Although scAT showed significant gene expression of *FAP*, no release of FAP from the tissue could be detected. The high fat meal increased postprandial circulating FGF21 but not FAP. Circulating levels of FAP but not FGF21 were significantly reduced after weight loss. On the other hand, *FAP* expression in scAT was increased. In conclusion, release from scAT does not appear to contribute to circulating concentrations of FGF21 and FAP and their responses to ingestion of a high fat meal or weight loss, respectively, in individuals with obesity.

Introduction

Fibroblast growth factor 21 (FGF21), a regulator of energy metabolism, is mainly expressed and secreted by the liver, but other tissues, such as white and brown adipose tissue, skeletal muscle and cardiac muscle, also express and secrete FGF21 under certain circumstances in humans [1–3]. Whether FGF21 from these tissues contributes to circulating FGF21 concentrations and under what conditions is less clear [1]. Plasma concentrations of FGF21 are increased in human obesity. Several studies have investigated the effects of diet-induced weight loss on circulating FGF21 concentrations after weight loss with inconsistent outcomes [4–8]. Whether changes in adipose tissue secretion of FGF21 contribute to changes in circulating FGF21 concentrations with weight loss is unknown. Human FGF21 is inactivated by fibroblast activation protein (FAP) [9]. FAP exists both on the cell surface and in a soluble, circulating form in the blood. The cellular origin of soluble FAP is unknown [10].

In this study we measured total FGF21, active FGF21, and FAP concentrations in arterialized blood and venous blood draining subcutaneous abdominal adipose tissue (scAT) in obese volunteers under fasting conditions, after a high-fat meal and before and after diet-induced weight loss.

Subjects and methods

Adult volunteers (BMI between 28 and 35 kg/m^2) were randomized to either a low calorie diet (LCD; 1250 kcal/d) for 12 weeks or a very low calorie diet (VLCD; 500 kcal/d) for 5 weeks. Both groups lost similar amounts of weight during this period. Subsequently, all participants returned to a weight maintenance diet and kept this for 4 weeks (weight stable period). Measurements were performed at T1 (baseline), T2 (end of the energy-restricted diet period), and T3 (end of weight stable period). Arterio-venous difference measurements were performed in 16 of the volunteers at T1 and T3, whereas microarray gene expression analysis in

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Table 1 Subcutaneous adipose tissue plasma flow, concentrations of total FGF21, active FGF21, and FAP in plasma from arterialized and subcutaneous adipose tissue venous blood, their arterio-venous differences and fluxes under fasting conditions before and after weight loss[a]

	Before weight loss (median ± IQR)	After weight loss (median ± IQR)	*P* value for difference before vs after
ATPF ((ml/min)/100 ml) (*)	0.69 ± 0.36	0.68 ± 0.38	0.940
Arterialized totFGF21 (pg/ml)	121.3 ± 190.6	111.2 ± 73.0	0.191
scAT venous totFGF21 (pg/ml)	120.5 ± 188.9	111.6 ± 73.4	0.211
a-v difference totFGF21 (pg/ml)	−1.7 ± 18.7	0.1 ± 5.4	0.609
totFGF21 flux ((pg/min).100 ml)	−1.3 ± 10.5	0.2 ± 3.0	0.865
Arterialized actFGF21 (pg/ml)	8.5 ± 51.0	10.4 ± 25.1	0.136
scAT venous actFGF21 (pg/ml)	19.8 ± 50.5	17.0 ± 22.8	0.064
a-v difference actFGF21 (pg/ml)	0.0 ± 13.6	0.0 ± 6.0	0.701
actFGF21 flux ((pg/min).100 ml)	0.0 ± 7.3	0.0 ± 3.6	0.382
Arterialized FAP (ng/ml) (*)	116.9 ± 28.4	100.5 ± 23.4	0.017
scAT venous FAP (ng/ml) (*)	116.2 ± 27.4	104.0 ± 21.9	0.100
a-v difference FAP (ng/ml) (*)	0.8 ± 12.1	−3.5 ± 11.0	0.279
FAP flux ((ng/min).100 ml) (*)	1.6 ± 9.0	−1.9 ± 6.4	0.248
Arterialized actFGF21 (%totFGF21) (*)	12.7 ± 14.2	11.5 ± 11.3	0.676
scAT venous actFGF21 (%totFGF21) (*)	16.8 ± 14.7	11.5 ± 9.1	0.055

ATPF adipose tissue plasma flow, *totFGF*21 total FGF21, *actFGF*21 active FGF21, *scAT* subcutaneous adipose tissue, *a-v* arterio-venous, *SD* standard deviation, *IQR* interquartile range

[a]Average of the values at $t = -30$ and 0 min; (*) mean ± SD

adipose tissue biopsies was performed at T1, T2, and T3. The study design and methods used for the measurements of arterio-venous differences across adipose tissue and the microarray analysis have been described in detail elsewhere [11–13].

Total FGF21 was measured via ELISA using a combination of three monoclonal antibodies: a mid-domain (amino acids 136–143), a C-terminal domain and a polyclonal anti-FGF21 detection antibody based on [14]. Active FGF21 was captured with the C-terminal specific antibody (custom mouse anti-FGF21 antibody developed by Morphosys/AbD Serotec) in combination with the same polyclonal anti-FGF21 detection antibody. FAP was measured with the FAP Human ELISA Kit (abcam (ab193701).

Data are presented as mean ± standard deviation (SD) for normally distributed variables or as median ± interquartile range (IQR) for nonnormally distributed variables (FGF21 concentrations) and appropriate parametric or nonparametric statistical tests were used.

Results

Fasting total FGF21 (totFGF21) and active FGF21 (actFGF21) were highly variable among individuals. TotFGF21 ranged from undetectable (<5 pg/ml; $n = 1$) to 490 pg/ml. ActFGF21 ranged from undetectable (<5 pg/ml; $n = 5$) to 170 pg/ml. For the data analysis the undetectable concentrations were set to zero. On average 15% of totFGF21 circulated in its active form.

There were no statistically significant differences in fasting concentrations of totFGF21, actFGF21, or FAP between arterialized and scAT venous plasma, neither before nor after weight loss (Table 1). The fluxes of totFGF21, actFGF21, and FAP across scAT were also not significantly different from 0 before or after weight loss (all $P > 0.20$).

Arterial and scAT venous concentrations of totFGF21 ($P = 0.002$ and $P = 0.001$, respectively) and actFGF21 ($P = 0.003$ and $P = 0.086$, respectively) but not of FAP, increased after consumption of the high-fat meal at baseline and similarly after weight loss (Fig. 1). There was no evidence for FGF21 or FAP secretion from scAT after consumption of the high-fat meal. Figure 1 also shows the concentrations of glycerol in arterialized and venous plasma supporting the adequacy of the arterio-venous balance technique.

After weight loss arterial and scAT venous plasma concentrations of totFGF21, actFGF21, or FAP were lower under fasting conditions (Table 1), but only the change in arterialized FAP concentrations was statistically significant ($P = 0.017$). Furthermore, mean concentrations of totFGF21 and actFGF21 over the 5-h test did not differ significantly from before weight loss. The mean arterialized and venous concentrations of FAP were significantly higher before than after weight loss ($P = 0.033$ and $P = 0.041$, respectively).

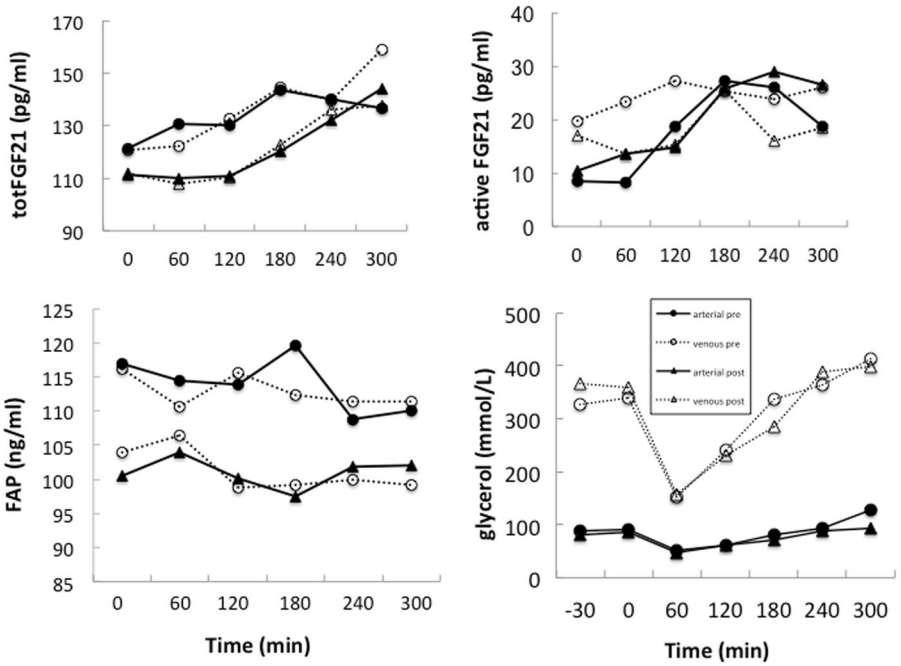

Fig. 1 Arterial and subcutaneous adipose tissue venous concentrations of total FGF21 (totFGF21) (median), active FGF21 (actFGF21) (median), FAP (mean), and glycerol (mean) at baseline ($t = -30$ and 0 min) and after consumption ($t = 60$ to 300 min) of a high fat mixed meal, before and after weight loss. Continuous lines and closed symbols arterialized concentrations, dotted lines and open symbols subcutaneous adipose tissue venous concentrations; circles before weight loss, triangles after weight loss

Gene expressions of *FGF21* and its receptor *FGFR1* were very low and did not pass the noise filtering. Gene expression of *FAP* was significantly increased after weight loss (fold change (FC) 1.18, $q = 0.021$), gene expression of *KLB* (ß-klotho) was not significantly changed (FC − 1.09, $q = 0.149$).

Discussion

The results demonstrate that FGF21 and FAP are not released from abdominal subcutaneous adipose tissue in individuals with obesity, neither before nor after weight loss nor after a high-fat meal. This is in agreement with undetectable gene expression of *FGF21* in this tissue under fasting conditions. Although scAT showed significant gene expression of *FAP*, no release of FAP from the tissue could be detected. Therefore, release from scAT does not appear to contribute to circulating concentrations of FGF21 and FAP and their responses to ingestion of a high-fat meal or weight loss, respectively. Furthermore, the high-fat meal increased postprandial circulating FGF21 but not FAP and the 10% weight loss did not affect fasting or postprandial FGF21 concentrations, whereas postprandial FAP was significantly reduced.

Release of FGF21 from human adipocytes in vitro has been reported [3], but studies in mice suggest that although *FGF21* is expressed in white adipose tissue and secreted from adipocytes, FGF21 acts locally in an autocrine or paracrine fashion instead of entering the circulation [1, 15]. In agreement with these data, our results indicate that, even if FGF21 is produced in human adipose tissue, it is not secreted into the circulation under the conditions studied.

The literature on the effects of meal ingestion on plasma FGF21 concentrations in humans is inconsistent [16–19]. Our data suggest a postprandial increase in plasma FGF21. Since none of these studies included a fasting time control arm, it cannot be excluded that circadian changes in FGF21 have interfered with these results [20]. Moreover, meal compositions were different in the different studies.

Weight loss did not affect fasting or postprandial circulating FGF21 levels in our study. Equivocal results have been reported: increases [4, 7], decreases [5, 7, 8], and unchanged levels [6] have been found. Differences in baseline concentrations of FGF21, reflecting differences in baseline characteristics, amount of weight loss and concomitant level and type of energy restriction, and the duration of weight loss may contribute to this variation.

Gene expression of *FGF21* was undetectable in scAT of our obese participants in agreement with other studies in humans, e.g. [16]. In contrast, other groups did report *FGF21* gene expression in human scAT, although without clear quantification [3, 4]. Although we cannot exclude that *FGF21* gene expression in scAT can be stimulated, expression appears to be extremely low after an overnight fast. In contrast, there was a clear expression of *FAP*, *KLB*, and *FGFR1* in human scAT indicating that FGF21 is likely to act in scAT and its activity can be regulated locally.

FAP gene expression in scAT increased after weight loss, but no release of FAP from scAT before or after weight loss was found. Plasma concentrations of FAP were reduced after weight loss, suggesting reduced FAP secretion from

other tissues than scAT or increased FAP clearance after weight loss.

In conclusion, human subcutaneous adipose tissue is not a source of circulating FGF21 or FAP in overweight and moderately obese individuals, neither before nor after weight loss or in response to a high fat mixed meal. Circulating levels of FAP, the enzyme that inactivates FGF21, were reduced after weight loss. On the other hand, *FAP* expression in scAT was increased, which may explain the tendency for lower scAT venous levels of active FGF21 after weight loss.

Author contributions MAvB and ECMM conceived the study and RGV and NJTR performed it. ACA and CCC were responsible for the analysis of FGF21 and FAP. MAvB wrote the manuscript. All authors commented on the content and approved the final version.

Compliance with ethical standards

Conflict of interest MAvB, ECM, RGV, and NJTR declare that they have no conflict of interest. CCC and ACA are employed by Eli Lilly and Company. This study was supported by the Netherlands Organisation for Scientific Research TOP, Grant Number: 200500001.

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